Abstract

Storm events and the corresponding runoff often result in a substantial increase in microbial and particulate solids loading to receiving waters. Microbial-particle interactions are important determinants of microbial fate and transport, as microbes associated with particles tend to settle out of waters, while microbes in the free phase may travel much greater distances downstream. These interactions also impact the effectiveness of detention basins for microbial removal from stormwater. The partitioning of several microbial species (fecal coliforms, *E. coli,* enterococci, *Clostridium perfringens* spores, and total coliphage) was investigated in urban stormwater runoff. Grab samples were taken under background conditions and during storm events, and samples were also taken throughout one storm event. The fraction of organisms associated with settleable particles was determined by microbial analysis both before and after samples were subjected to a centrifugation process. Centrifugation was shown to remove more than 97% of high density (> 2.5 g/cm^3) particles, while retaining the majority of low density (1.05 g/cm^3) particles that were representative of "free phase" microbial organisms.

Storm samples were characterized by increased microbial and particle concentrations relative to background conditions. The fraction of organisms removed via centrifugation varied by microbe and between background and storm conditions. The highest average removals were observed in fecal coliforms (28% background; 58% storm) and *Cl. perfringens* spores (45% background, 61% storm), whereas total coliphage demonstrated the lowest removals (13% in both background and storm conditions).

Particle concentration is consistently high throughout storm events, but peak microbial concentrations tend to vary by microbe. Fecal coliforms tend to be highest as flows are increasing, *E. coli* concentrations tend to be highest near peak flows, and enterococci and total coliphage concentrations tend to be highest as flows are declining.

Introduction

Nonpoint source water pollution is a water quality problem of increasing concern. It is a leading cause of water pollution in the United States, and urban nonpoint source pollution is a major factor in freshwater and estuary impairment (EPA, 2002). The Clean Water Act Amendments of 1987 have sought to address the problem of nonpoint source pollution, but the identification and remediation of these sources has been much more difficult than for point sources (James, 2003). Nonpoint source contaminants can include suspended solids and a host of organic and biological contaminants. Of particular interest to public health are the microbial contaminants that enter watersheds in stormwater runoff. Pathogen contamination is the second most prevalent cause of waterbody impairment in the United States, with over 7,000 impairments nationwide (EPA, 2004). Storm events and the resulting runoff cause a substantial portion of the contaminant loading of microbial organisms to receiving waters (Crabill et al 1998, Nix 1994, Wanielista et al. 1977, Weibel et al. 1964). Bacterial loadings in urban streams have been linked to housing density, population, and development (Young and Thackston 1999), and the threats to water quality will continue to rise with urban growth.

Microbial contamination can have widespread public health consequences, including adverse effects on drinking water, recreational water sources, aquatic food, and watersheds. Recent research suggests a possible link between rainfall runoff and outbreaks of waterborne disease (Curriero et al. 2001, Rose et al. 2001). Some studies have addressed the post-storm accumulation of microbes in sediments and their subsequent impacts on activities such as shellfish consumption, recreational water contact, and sediment dredging (Haile et al. 1999, Sunen and Sobsey 1999, Crabill et al. 1998, Grimes 1980). While it is understood that microbes are mobilized by stormwater runoff and that many of these microbes are found in the

sediments of water bodies, less in known about microbial behavior in the water column and the transport processes by which microbes become part of the sediment. Improved knowledge of microbial partitioning can provide a basis for better understanding these transport processes.

Microbial-particle interactions can have critical effects on microbial fate and transport. Microbes may associate with particles in the water column or remain in the "free" phase. Microbes associated with particles, particularly heavier and/or inorganic particles, will tend to settle out of the water column more quickly, while microbes in the free phase may travel much greater distances downstream. In addition, microbes associated with particles often tend to survive longer in natural waters (Howell, J. M. et al. 1996, Sherer et al. 1992, Burton et al. 1987, Gerba and Schaiberger 1975). Partitioning of microbes therefore affects not only microbial fate and transport in the environment, but also the length of time these organisms remain a potential threat to public health.

This study characterizes the partitioning of several microbial species (fecal coliforms, *E. coli*, enterococci, *Clostridium perfringens* spores, and total coliphage) in urban stormwater runoff by investigating potential links between microbial concentration, particle concentration and different particle size classes. Such partitioning relationships are essential for modeling the location and severity of water quality impairment, as well as for determining the effectiveness of sedimentation as a treatment strategy (i.e. detention basins). Many current water quality models (e.g. BASINS) assume that all microbes are in the free phase, an error in the case of many microbes that impacts water quality estimates. Given that models are currently a primary means of evaluating permit applications (e.g. National Pollutant Discharge Elimination System) and new regulatory initiatives (e.g. Total Maximum Daily Loads,

Stormwater Management), research that facilitates improved modeling through consideration of microbial partitioning may have a significant impact on a range of policy issues.

Background

Research has described the partitioning relationships between particles and various contaminants in the water column, both organic (Stenstrom et al. 1984, Hunter et al. 1979) and inorganic (Characklis and Wiesner 1997, Tanizaki et al. 1992). However, much less work has been done to characterize the partitioning relationships of microbial contaminants. Previous particle-microbial studies have focused on the sediments rather than the water column (Sherer et al. 1992, Burton et al. 1987, LaBelle and Gerba 1980). While this information is important in evaluating the possible impacts of resuspension, it is less useful for evaluating partitioning in the water column. This is important because it is the partitioning behavior in the water column that plays a critical role in determining microbial fate and transport.

Some studies have observed and reported evidence of particle-microbial relationships in the water column and in sediments, with attention usually focused on fecal coliforms. Gerba and Schaiberger (1975) indicated that bacterial contaminants associate with particles in stormwater runoff, and Gannon (1983) indicated that samples with high turbidity coincided with high fecal coliform levels. Other studies have observed partitioning of viruses in the water column (Gerba and Schaiberger 1975) and sediments of estuarine environments (LaBelle and Gerba 1980), but viral partitioning has not been investigated in fresh water conditions. Although some trends have been observed, few of the previous studies have quantitatively characterized the partitioning relationships in a manner useful for estimating transport and/or removal processes. In addition, the relationships between particles and other microbial organisms (e.g. *E. coli* and enterococci) have not been developed.

Characterizing the partitioning of particular microbes to particles in different size classes can create a better understanding of the fate and transport of microbial contaminants.

Larger, denser (e.g. inorganic) particles will have greater settling velocities. Microbes attached to such particles will not remain suspended in the water column as long and thus will travel shorter lateral distances in receiving waters than those associated with less dense (e.g. organic) particles or those remaining in the free phase.

A study by Schillinger and Gannon (1985) investigated the possibility of a general association between fecal coliforms and particles. Single stormwater grab samples were collected during storms events from a storm drain and immediately analyzed for turbidity, suspended solids, total solids, fecal coliforms, *Klebsiella* bacteria, *Pseudomonas* bacteria, and Gram negative bacteria. A suggestive positive trend between fecal coliforms and suspended solids concentrations was observed in the raw samples, although the trend was not statistically significant. A partitioning relationship is suggested by this data, but there were no direct particle size measurements of the suspended solids.

Samples were collected and some were settled. The supernatant of the settled samples was removed and tested for bacteria. The total particle mass of the supernatant and sediment was measured. In the bacterial samples, 16.8% of the fecal coliforms, 33% of the *Klebsiella,* and 31.1% of the *Pseudomona* settled out of the samples. In general, the *Klebsiella* and *Pseudomonas* bacteria settled to a greater extent than fecal coliforms. These results suggested that the degree of microbial partitioning may vary by organism.

Stormwater particles were separated into four size fractions by filtration. Most of the bacteria associated with the particles retained by the larger-sized filters (30 and 52μm). Fecal coliforms had the lowest percent retention of the four bacteria investigated. In all filtration experiments, at least 50% of bacteria were not removed by a filter with a 5 μm cutoff, indicating that a large percentage of these organisms were in the free phase (or associated with

very small particles). Because particles and microbes smaller than 5μm could have been retained by the filter cake that builds up during filtration, the fraction existing in the free phase may have been underestimated. The study also noted that the shear stresses associated with the smaller filter screen could have caused problems with the data.

Bacterial partitioning has also been explored in a constructed wetland and a water pollution control pond system (Davies and Bavor, 2000). Analysis was completed for presumptive thermotolerant coliforms, enterococci, total heterotrophic bacteria, and *Clostridium perfringens* spores. Inflow and outflow water samples were collected weekly. Sediment and water column samples were collected once. The particle size distribution of the sediment samples was determined using the pipette method (the particle size distribution of the water column was not considered). A set of column experiments were conducted with these sediments resuspended in deionized water. For the pipette method, settling velocities, derived via Stokes' Law, were used to determine the sampling time for each of the six size fractions (for a total particle range of <2 μm to >62 μm) at a certain depth in the sample. The sediments were resuspended in deionized water and allowed to settle, with samples acquired at specified times by pipette at the set depth. The study concluded that the bacteria were predominantly adsorbed to fine clay particles (less than 2 μm in diameter). However, since resuspended sediments were used to perform this analysis, the initial behavior in the water column was not directly investigated.

These previous studies have focused on grab samples to characterize storm events. Schillinger and Gannon collected samples at peak flow, and Davies and Bavor collected weekly samples. While both studies have made initial steps in linking storm events and

microbial concentrations, neither explored how microbial concentrations and partitioning behavior might change throughout a storm event.

Research indicates the difficulty in characterizing a "typical" storm (Field et al. 1993, Qureshi and Dutka 1979, Weibel et al. 1964, Wanielista et al. 1977, Geldreich et al. 1968). Variations in contaminant concentrations and receiving water characteristics are a function of pre-storm conditions (e.g. time since last storm), storm duration, stream flow, water temperature, and other site characteristics. The first flush refers to the first portion of stormwater that enters a water body and is often thought to have the majority of microbial contaminants associated with the storm event. Qureshi and Dutka (1979) determined that peak contaminant concentrations can vary considerably and are not necessarily found in the first flush. No apparent links between peak flow and the occurrence of peak microbial populations were observed. Due to the changes in particle and microbial concentrations throughout the duration of a storm event, it may be more appropriate to use a combination of single grab samples and samples collected over the hydrograph (intra-storm samples) to provide a more accurate means of characterizing the total microbial load from a storm event.

Earlier studies have taken initial steps in developing the relationships describing bacterial-particle interactions in stormwater. Both Schillinger and Gannon (1985) and Davies and Bavor (2000) have suggested that a considerable fraction of bacteria associate with suspended particles, but quantification of these relationships is still lacking. In addition, the partitioning relationships of other microbes, including protozoan and viral organisms, have yet to be investigated in the water column of fresh waters. Characterizing the partitioning of bacterial, protozoan, and viral organisms in stormwater will provide valuable information for

improving estimates of microbial transport and determining the effectiveness of stormwater management strategies.

Methods

3.1 Site Selection

Samples were collected from three locations: Eno River, Meeting of the Waters Creek, and Booker Creek, all in and around Chapel Hill and Durham, North Carolina. Booker Creek and Meeting of the Waters Creek flow into Jordan Lake, which then flows into the Cape Fear River. The Eno River flows into Falls Lake, which then flows into the Neuse River. Sites were selected to be representative of different types of land use.

Samples from the Eno River were collected at the Guess Road crossing of the river. The surrounding land is classified as low density residential. There is a USGS gage station downstream at the SR 501 crossing of the river, and the Town of Durham also collects water quality data at this location.

Meeting of the Waters Creek was sampled at the bridge leading to the entrance to the Orange Water and Sewer Authority (OWASA) Mason Farm Road Wastewater Treatment Plant. This location was upstream of the OWASA discharge. The land use is classified as primarily institutional; the University of North Carolina at Chapel Hill is upstream. Water quality is monitored monthly at this location by the Town of Chapel Hill and a USGS gage station is located just downstream.

Booker Creek was sampled at Willow Drive, downstream of the Eastgate Shopping Center. The land use is classified as commercial and residential, and monthly water quality data is collected by the Town of Chapel Hill. There is no flow information available for the creek.

3.2 Sample Collection

Samples were collected in background (dry) and wet weather conditions, with background conditions defined as at least three days without appreciable precipitation. Storm sampling events were characterized by at least three days without appreciable rainfall before the event and an increase in stream flow of at least four times pre-storm flow. All samples were collected in rinsed new, sterile cubitainers. Samples were collected in duplicate for quality control and reproducibility purposes. Samples were obtained either by wading into the stream and submerging the container in the stream water, or by lowering a bucket into the stream when the water level or bridge height would not allow for wading. Water temperature was recorded at the time of sampling and pH was determined in the laboratory (Accumet 10 pH meter). After samples were collected, they were stored in coolers with ice packs and transported to the laboratory for immediate analysis.

During dry weather, background grab samples were collected. For wet weather samples, 4-6 storms were characterized by single grab samples (depending upon sampling site) and 1 storm was characterized by intra-storm sampling (at two sites). This intra-storm sampling consisted of 5-6 samples taken over a 24-hour period to analyze the conditions over the hydrograph.

3.3 Sample Partitioning

Centrifugation was used to remove particles and associated microbes from suspension. Control experiments (Section 3.5) characterized the degree to which centrifugation removes the larger and/or denser particles. By using centrifugation, microbial and particle concentrations in a raw and settled sample can be compared in order to better understand

particle-microbial partitioning behavior. Centrifugation rather than filtration was used because of the potential problems of clogging the filters or shearing particles (Schillinger and Gannon, 1985).

Once the samples were returned to the laboratory, any material that may have settled during transport was resuspended by gently inverting each cubitainer three times. A portion of each sample (cubitainer) was centrifuged in 1 L autoclaved centrifuge bottles. A Sorvall RC-3 H-6000A Centrifuge was used to separate the samples into the two size fractions. The centrifuge was run at 1164 x g (2000 rpm) for 10 minutes with a brake of 4 while a temperature of 4 °C was maintained.

Once centrifugation was complete, approximately 700 mL of supernatant from each centrifuge bottle was removed. The apparatus used to remove the water from the centrifuge bottles consisted of a vacuum flask with a hose connected to a pipette that that drew water out of the centrifuge bottle and into the flask. The bore of the 5 mL pipette was enlarged at both ends to create a wider aperture and limit particle breakup from sheer forces. The pipette tip was kept just under the water surface on the edge of the bottle as the water was withdrawn. The samples for particle analysis were put in 1 L sample bottles that had been washed with tap water three times and deionized water three times. The samples for microbial analysis were put in 1 L autoclaved sample bottles. After removal, all raw and centrifuged samples were stored in coolers. All equipment was then cleaned three times each with tap water, 10% bleach, and deionized water prior to processing the next sample.

3.4 Physical Analysis

The particle counting and size distribution was done following Standard Method 2560. A Met-1 particle counter (light blockage instrument) was used to determine particle

concentration (#/100mL) and particle size distribution. The measurable size range of the instrument was 5 to 100 μm, with individual channel widths of 0.5μm. Again, in order to counteract any settling that occurred during transport, each sample was gently agitated by inverting the bottle three times prior to analysis.

Total organic carbon analyses were done following Standard Method 5310B using a Shimadzu TOC-5000 Combustion-Infrared instrument. The calibration standards were prepared using potassium hydrogen phthalate. Samples were acidified with hydrochloric acid and purged with nitrogen gas to remove inorganic carbon. Total suspended solids were evaluated using Standard Method 2540D.

3.5 Control Experiments

Control experiments were performed to indicate the effectiveness of centrifugation on removing contaminants from natural waters. Since contaminant size and density affect sedimentation, surrogates for microbes and inorganic particles were used to investigate their settling behavior. Latex particles (10 μm nominal diameter, 1.05 g/cm^3 density) were used as a surrogate for microbes (see Table 1). Latex particles may also act as a reasonable surrogate for organic particles. Glass beads (5-50 μm size range, 2.5 g/cm^3 density) were used as a surrogate for inorganic particles. Results using the 10 μm latex particle standard solution indicate that these 10 μm latex particles are not effectively removed by centrifugation (Figure 1). This suggests that the majority (71%) of free phase microorganisms and many organic particles will remain in solution following the prescribed centrifugation procedure. The sample analysis on the standard solution of glass particles indicates that the vast majority (>97%) are removed by the described centrifugation procedure (Figure 2). Removal of 6 μm glass beads was approximately 50%, and glass beads greater than 10 μm experienced at least

90% removal. While the results of these experiments are not definitive with respect to all of the states in which the microbes of interest may exist in natural waters, they provide strong evidence that the described centrifugation regimen removes the majority of organisms associated with inorganic particles, while leaving most of the free phase organisms or those attached to light organic particles in suspension. The distinction between the denser particles and the lighter particles/free phase organisms is, of course, the critical factor when estimating transport or removal efficiencies.

Table 1: Physical Characteristics of Select Microbes and Particles

	Equivalent Spherical Diameter (µm)	Density (g/cm3)
Inorganic Particles		~2.6[1]
Organic Particles		~1.2[1]
Microbes		
Fecal Coliforms	1-4[2]	
E. coli		1.09-1.13[3]
K. *aerogenes*	1.06[4]	1.017[4]
Coliphage		
Coliphage MS-2		1.33-1.46[5] in CsCl
Protazoa		
Cryptosporidium	4-6[6]	1.06[7]
Viruses	0.02-0.3[6]	
Norwalk virus		1.39-1.40[8] in CsCl

Note: 1 Chapra, S. C., 1997
2 Linsley, R. K et al., 1992
3 Bratback, G. and I. Dundas, 1984
4 Sharma et al., 1993
5 Rohrmann, G. F. and R. G. Krueger, 1970
6 American Water Works Association, 1999
7 Metge, D. W., 2003
8 USFDA, 2003

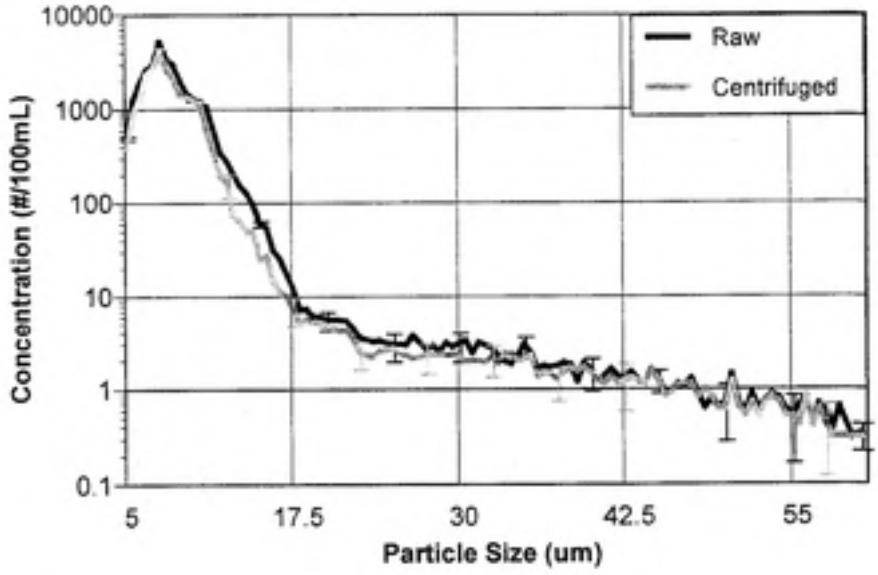


Figure 1: 10µ Latex Standard

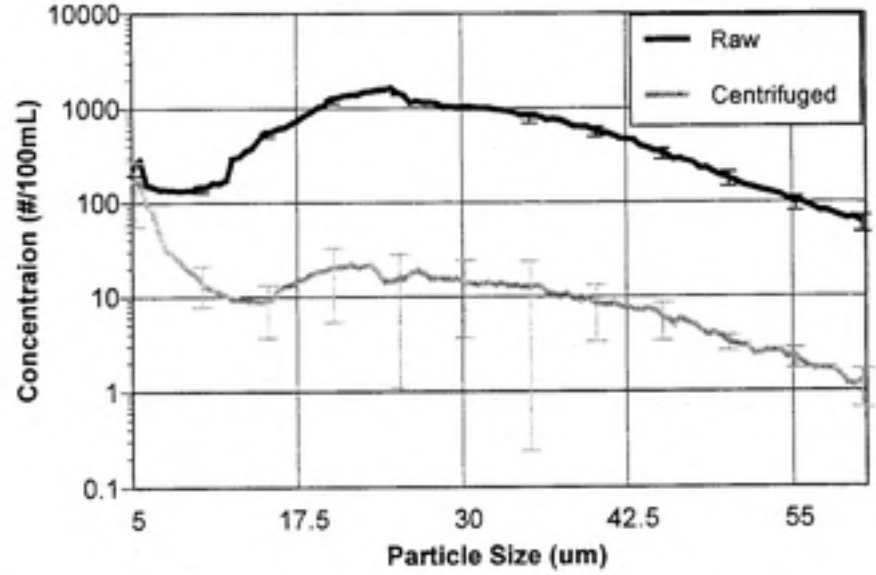


Figure 2: Glass Standard

3.6 Microbial Analysis

Microbial analyses were conducted within 24 hours of sample collection. Samples were analyzed for fecal coliforms *E. coli*, enterococci, *Clostridium perfrigens* spores, and total

coliphage. Fecal coliforms, *E. coli*, and enterococci are used as indicators for bacterial pathogens, *Cl. perfringens* spores are used as indicators for protozoan parasites, and total coliphage are used as indicators of viral pathogens. Sample waters were diluted in order to obtain microbial concentrations within the measurable range of each analysis.

Analyses for fecal coliform bacteria and *E.* coli were completed simultaneously using Colilert®, a biochemical analytical test developed and outlined by IDEXX (IDEXX, Westbrook, Maine) and described in Standard Methods. The Colilert® method, generally used to detect total coliform bacteria and *E. coli*, was modified for detection of thermo-tolerant fecal coliform bacteria and *E. coli* by incubating samples at the elevated temperature of 44.5°C (Chihara et al. 2004, Yakub et al. 2002). Coliform and *E. coli* concentrations were determined using the Quani-tray® system provided by IDEXX. This system produces a series of positive and negative results on each tray from which a Most Probable Number (MPN) of colony forming units per 100 mL sample (MPN/100 mL) is determined with accompanying 95% confidence interval based on a logarithmic Poisson distribution.

Analyses for enterococci were performed using Enterolert™(IDEXX), a biochemical test that has been shown to be effective in determining enterococci concentration in drinking water and wastewater (Simmons et al. 2004, Yakub et al. 2002). MPNs and confidence intervals for enterococci were determined using the same Quani-tray® plate system. Values are reported as MPN/100mL. For both Colilert® and Enterolert™, analyses were performed using two trays, doubling the sample size, in order to reduce confidence intervals.

Clostridium perfrigens spores were enumerated using a multiple fermentation tube technique. In order to detect only the spores, environmental samples were heated to 65°C for 20 minutes to inactivate vegetative bacteria before appropriate sample dilutions were

inoculated into iron milk media tubes. Inoculated tubes were incubated at 41°C for 24 hours. A MPN was determined based on stormy fermentation of positive samples in the fermentation tubes and are reported as MPN/100mL.

Total coliphage concentrations were enumerated using a modified single agar layer method described as the *Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure* (USEPA, 2000). In order to detect total coliphage, instead of only somatic coliphage as specified in the method, *E. coli* C-3000 was used as the host bacteria. Briefly, 100 mL volumes of sample were added to tryptic soy agar, supplemented with magnesium chloride ($MgCl_2$), allowed to solidify in petri dishes on the benchtop, and then incubated overnight at 37°C. The following day, clear zones of lysis, termed plaques, were enumerated to determine the total coliphage concentrations in the sample environmental waters. Values are reported as plaque forming units per 100ml (PFU/100mL).

3.7 Analytical Error

The analytical error associated with the measurement techniques can be significant. Therefore, in order to avoid confusion between analytical error and natural variability in the storm and background samples when presenting results, a full description of analytical error is undertaken here. Mean values are reported with 95% confidence intervals with normal distributions for particle concentration, TSS, and TOC. As previously stated, all MPNs are reported with 95% confidence intervals that have logarithmic Poisson distributions. To limit analytical error in the physical analysis, all analyses except those for *Cl. perfringens* spores and total coliphage were performed in duplicate. This increased the number of wells used to determine the MPN in the IDEXX analyses (fecal coliforms, *E. coli*, and enterococci) and

reduced the confidence intervals approximately 30% as compared to single-tray analyses. Graphs of the analytical error that results from the respective microbial tests are shown in Figures 3-5. It should be noted that only MPNs and variability in MPNs attributable to natural fluctuations (not analytical error) in storm and background samples are presented in Results. Values for each parameter across sampling events are reported as means, with error represented by plus or minus one standard deviation.

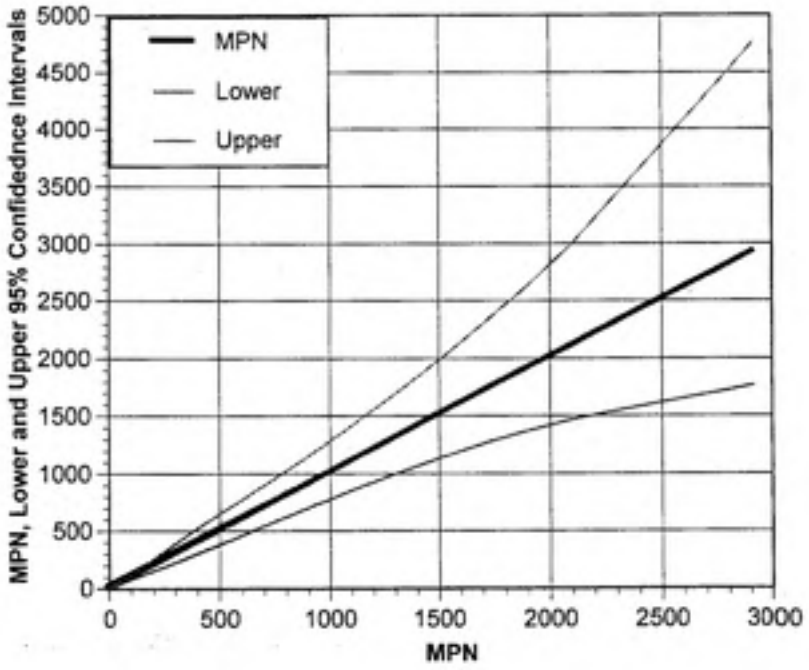


Figure 3: MPN and Lower and Upper 95% Confidence Interval for Fecal Coliforms, *E.coli*, and Enterococci

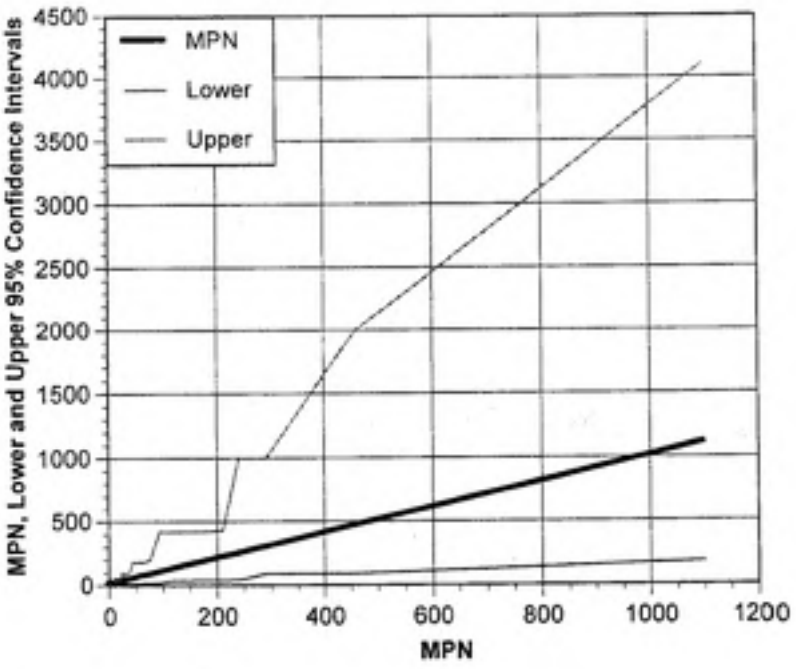


Figure 4: MPN and Lower and Upper 95% Confidence Interval for *Cl. perfringens* Spores

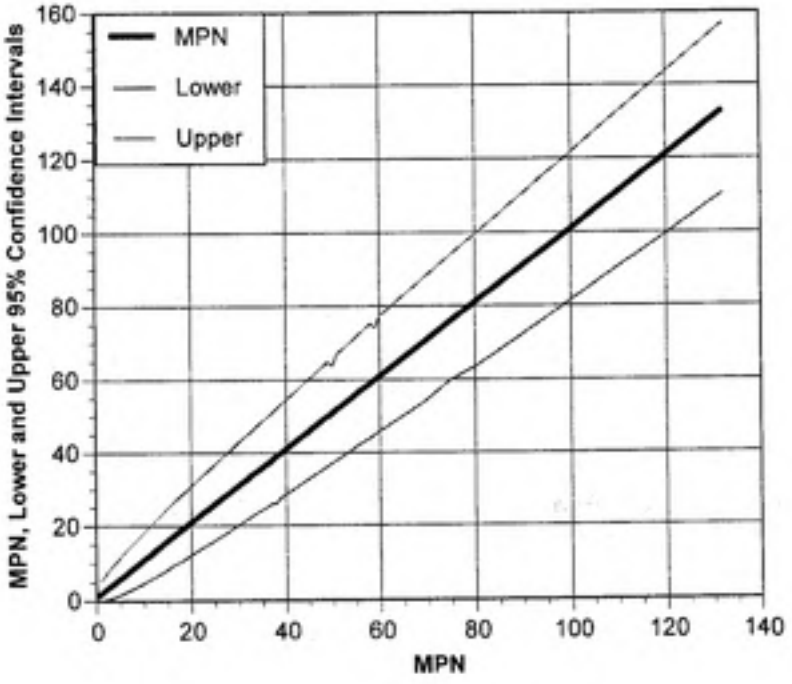


Figure 5: MPN and Lower and Upper 95% Confidence Interval for Total Coliphage

Results and Discussion

Results are presented in two sections: grab sampling and intra-storm sampling. The grab samples were obtained under both background and storm conditions, and describe a step change in water quality. Results from the intra-storm sampling illustrate more continuous changes in water quality parameters throughout the progression of a storm event, while also allowing for calculations of the total contaminant loading occurring as a result of a storm.

4.1 Grab Sampling

4.1.1 Grab Sampling Results

A distinct increase in the mean particle number concentration is observed between background and storm conditions across all three sampling locations (Figure 6), with storm samples showing greater variability. In Figure 6, mean background and storm particle size distributions are shown. Along with high and low values that correspond to the highest and lowest particle concentrations observed under all sampling events for background and storm conditions. There is a sharp increase in particle concentration across all size ranges as a result of stormwater runoff, although storm samples show greater variability than background samples. Storm variability is consistent with previous studies (Field et al. 1993, Qureshi and Dutka 1979, Weibel et al. 1964, Wanielista et al. 1977, Geldrich et al. 1968) that indicate that there is no "typical" storm.

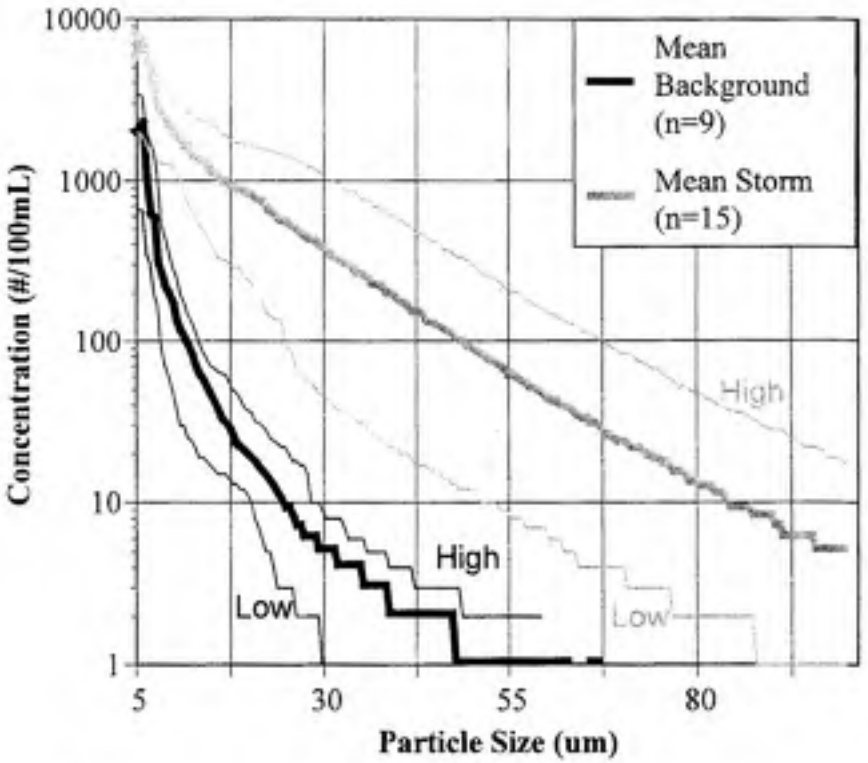
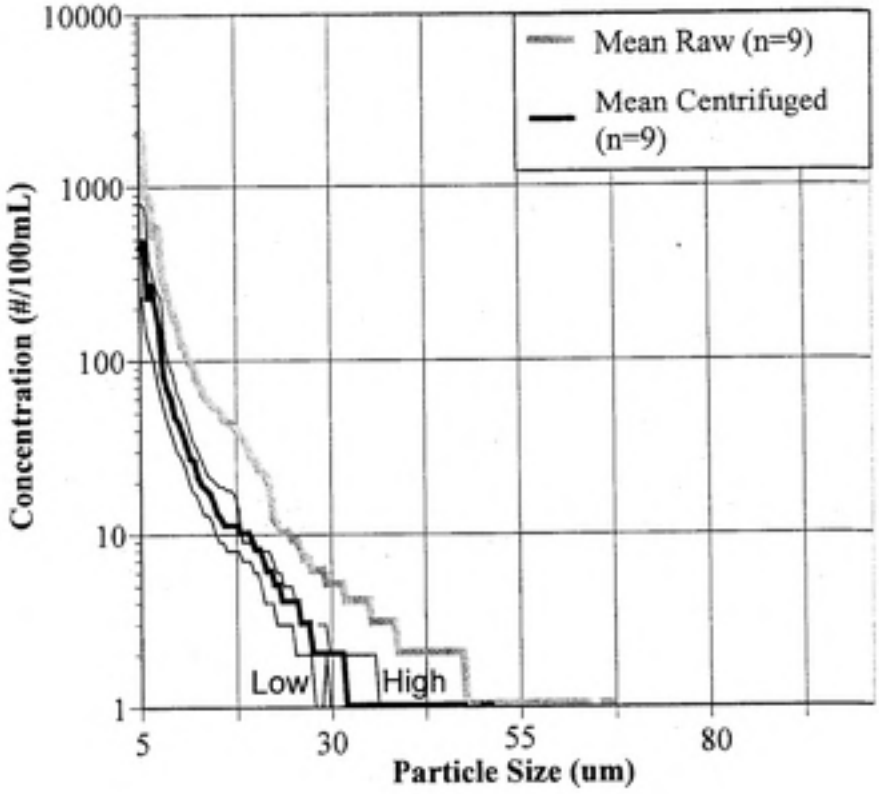


Figure 6: Particle Size Distribution in Background and Storm Samples

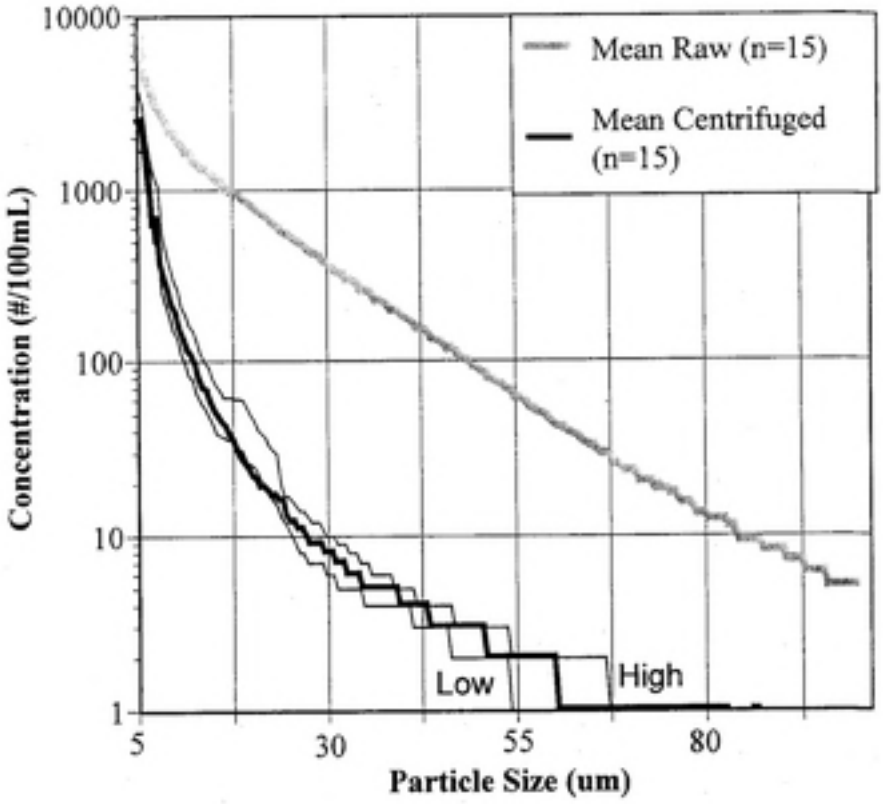
Centrifugation removes a significant fraction (79% mean removal) of particles of all sizes under both storm and background conditions (Figures 7 and 8). Raw and centrifuged particle size distributions are shown in each figure, with the difference between the distributions representing the heavier particles removed via centrifugation. Centrifugation is more effective in removing particles in the storm samples, removing approximately 88% vs. 70% of the particles (5 μm to 100 μm) in the background samples. Standards analysis would suggest that the majority (>97%) of the heavier particles greater than 5μm are removed, and therefore, the majority of what remains in the suspension is likely to be lighter, organic particles, free phase microbes, or an amalgam of both. The presence of microbial and physical parameters is summarized in Table 2. The similar TOC concentrations in the raw and

centrifuged samples suggest that organic particles are remaining in suspension despite centrifugation or that all TOC is dissolved organic carbon (DOC).



Note: Low and high values for raw samples are given in Figure 6

Figure 7: Particle Size Distribution in Raw and Centrifuged Samples under Background Conditions



Note: Low and high values for raw samples are given in Figure 6

Figure 8: Particle Size Distribution in Raw and Centrifuged Samples under Storm Conditions

Table 2: Average Concentration of Microbial and Physical Parameters

		Fecal Coliforms (CFU/100mL)	*E. coli* (CFU/100mL)	Enterococci (CFU/100mL)	*Cl. perfringens* spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration* (#/100mL)	TSS (mg/L)	Settled TOC** (mg/L)
Eno River									
Background	Raw	893 ± 379	63 ± 52	45 ± 41	53 ± 72	7 ± 6	18,664 ± 6,071	6 ± 2	3.5 ± 1.4
(n=3)	Centrifuged	651 ± 259	66 ± 61	36 ± 29	45 ± 66	10 ± 8	4,514 ± 1,360	3 ± 3	3.4 ± 1.1
Storm	Raw	8,863 ± 8,995	2,058 ± 1,499	5,788 ± 8,565	244 ± 215	23 ± 15	92,884 ± 28,715	61 ± 68	4.4 ± 1.7
(n=5)	Centrifuged	2,662 ± 922	1,220 ± 736	2,772 ± 3,850	50 ± 41	21 ± 14	8,032 ± 4,283	10 ± 8	3.9 ± 1.0
Meeting of the Waters Creek									
Background	Raw	4,504 ± 4,212	208 ± 151	206 ± 105	32 ± 20	15 ± 8	10,342 ± 363	5 ± 2	2.9 ± 0.0
(n=3)	Centrifuged	3,037 ± 2,508	324 ± 257	182 ± 39	18 ± 6	9 ± 5	2,893 ± 616	2 ± 1	3.0 ± 0.0
Storm	Raw	11,784 ± 9,307	1,883 ± 1,258	2,776 ± 1,771	472 ± 340	46 ± 49	107,969 ± 25,208	127 ± 129	4.8 ± 1.1
(n=6)	Centrifuged	6,697 ± 4,074	1,305 ± 806	1,764 ± 897	326 ± 413	38 ± 37	18,343 ± 14,905	19 ± 12	4.3 ± 0.6
Booker Creek									
Background	Raw	1,233 ± 582	523 ± 438	134 ± 87	42 ± 44	22 ± 19	5,973 ± 3,861	5 ± 4	4.8 ± 1.3
(n=3)	Centrifuged	939 ± 493	330 ± 354	115 ± 78	10 ± 11	22 ± 16	2,145 ± 783	3 ± 3	5.2 ± 1.3
Storm	Raw	39,982 ± 67,338	7,937 ± 12,242	5,575 ± 5,782	725 ± 442	27 ± 19	106,038 ± 33,208	95 ± 103	6.1 ± 2.0
(n=4)	Centrifuged	15,426 ± 22,208	6,804 ± 10,576	3,899 ± 5,563	193 ± 232	33 ± 26	9,375 ± 4,877	9 ± 7	6.0 ± 2.7

Note: Values shown as mean ± standard deviation

* Measurable size range: 5 μm to 100 μm

* * Samples remain in the autosampler for some period prior to analysis, potentially allowing organic particles to settle out

Average microbial and physical characteristics of the raw and centrifuged samples are measured under both storm and background conditions. Values represent average concentrations measured across multiple sampling events, with accompanying standard deviations. The data for each individual sampling measurement with accompanying analytical error confidence intervals are shown in Appendix 1.

As might be expected on the basis of particle concentration data, TSS concentration increases by approximately 90% during storms at all three sites. Variability of TSS in storm samples is large, but the mean values are consistently greater than those observed in the background samples.

Mean concentrations of microbial organisms generally increase at all sites as a result of a storm event. Some microbial concentrations increase ten-fold or greater during storms, with the greatest increase observed in fecal coliform concentrations at Booker Creek. One particularly high background concentration of fecal coliforms resulted in a high mean background concentration at Meeting of the Waters Creek, but historical data from the Town of Chapel Hill (unpublished water quality monitoring data, Town of Chapel Hill, NC) suggests that this site is subject to considerable variability, and the high value observed was within this range.

The percentage of fecal coliforms as *E. coli* varies by site and with conditions (i.e. background or stormwater). For Eno River and Meeting of the Waters Creek, a greater percentage of fecal coliforms are attributed to *E. coli* under background conditions (Table 3). For all samples except the Booker Creek background sample, the percentage of fecal coliforms as *E. coli* was greater in the supernatant of the centrifuged samples than in the

raw samples. Fecal coliforms as a whole tend to be more effectively removed than *E. coli*, suggesting that non-*E. coli* fecal coliforms may be more likely to associate with inorganic particles. This would be consistent with Schillenger and Gannon's study (1985) that indicated *Klebsiela*, a non-*E. coli* fecal coliform, tended to have a greater degree of particle association than the fecal coliform group as a whole.

Table 3: Fecal Coliform and *E. coli* Concentrations

		Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	*E. coli*/FC
Eno River				
Background	Raw	893	63	7.1%
	Settled	651	66	10.1%
Storm	Raw	8,863	2,058	23.2%
	Settled	2,662	1,220	45.8%
Meeting of the Waters Creek				
Background	Raw	4,504	208	4.6%
	Settled	3,037	324	10.7%
Storm	Raw	11,784	1,883	16.0%
	Settled	6,697	1,305	19.5%
Booker Creek				
Background	Raw	1,233	523	42.4%
	Settled	939	330	35.1%
Storm	Raw	39,982	7,937	19.9%
	Settled	15,426	6,804	44.1%

Variability in microbial concentrations and the relative increase in storm vs. background concentration are the largest in the Booker Creek storm samples. This site is located downstream of a shopping plaza with substantial impervious land cover (e.g. parking lots), and stormwater runoff appears to have the greatest impact on water quality at this sampling location. Previous studies also support the idea that commercial land use can have a significant impact on stormwater quality (Tanizaki 1992, Schillinger and Gannon 1985, Qureshi and Dutka 1979, Weibel 1975).

In almost all cases, centrifuged samples have lower microbial, particle number, and TSS concentrations than the corresponding raw samples. Exceptions take place primarily when concentrations are low (background *E. coli* and total coliphage) and with discrepancies that are usually within the range of analytical uncertainty (see Section 3.7). It is also interesting to note that fecal coliforms, enterococci, and *Cl. perfringens* spores tend to be less effectively removed via centrifugation in the background samples than in the storm samples. This suggests that some microbes may associate with particles to a greater extent during storm events when particle concentrations are substantially higher.

Grab sample concentration data are important for gaining insight into the magnitude of microbial and sediment loading attributable to stormwater runoff, while analysis of the partitioning behavior is important for understanding fate and transport. Measurements of the fraction of each organism removed via the centrifugation process provide an indication of how effectively these microbial contaminants may be removed by settling. Data is provided for the fraction removed at Eno River (ER), Meeting of the Waters Creek (MWC), and Booker Creek (BC).

The fraction of TSS removed has particular implications for the effectiveness of detention basins in removing sediment. The fraction of TSS removed increases in the storm samples as compared to the background samples (Figure 9). The box and whisker plots, in addition to displaying the mean and median values, also indicate the 10% and 90% values by the whiskers and the 25% and 75% values by the lower and upper bounds of the box. The increased removal efficiency may be due to the increased prevalence of inorganic particles in storm samples and is consistent with particle removal data in storms and background samples (Figures 7 and 8).

The fraction of each microbe removed via centrifugation provides an indication of the degree of microbial partitioning to heavier particles (Figures 10 and 11). All of the organisms analyzed show some evidence of partitioning, although the degree of association varies by microbe. For most organisms, the partitioning behavior also varies between the background and the storm samples.

Fecal coliforms and enterococci demonstrate similar partitioning behavior. On average, 50% of fecal coliforms and 41% of enterococci in storm samples are removed via centrifugation. The fraction of all fecal coliforms removed in the storm samples increases relative to the background samples at all three sites. A similar trend is observed for enterococci at two of the three sites (Eno River and Meeting of the Waters Creek). The mean fraction of fecal coliforms removed in the background samples was approximately half of that under storm conditions. The increased removal in the storm samples suggests that the level of microbial-particle association increases during storm events.

The opposite relationship is observed with *E. coli*, where the fraction removed decreases 55% with increasing particle number concentration (i.e. during storms) although there is considerable variability. One interpretation of this might be that *E. coli* preferentially partitions to smaller particles, since the storm samples were characterized by larger relative increases in concentration in the larger size ranges. However, additional research would be needed in order to support a conclusive statement.

A similar trend is not readily observed for *Cl. perfringens* spores. The fraction removed appears relatively constant under background and storm conditions, with the exception of background concentrations at the Eno River. The fractions of *Cl.*

perfringens spores removed via centrifugation were relatively high (mean value of 60% across all sites) in almost all samples, indicating that these organisms were more frequently associated with particles than any of the others analyzed.

Total coliphage removal is more variable than that of fecal coliforms, *E. coli*, and enterococci. There is no consistent pattern of behavior of the fraction of total coliphage removed in the background vs. storm samples. In addition, 10 of the 24 samples had higher total coliphage concentrations in the centrifuged samples than in the corresponding raw samples. The high level of relative variability may be a result of the low concentrations of total coliphage and the uncertainty associated with the analytical techniques.

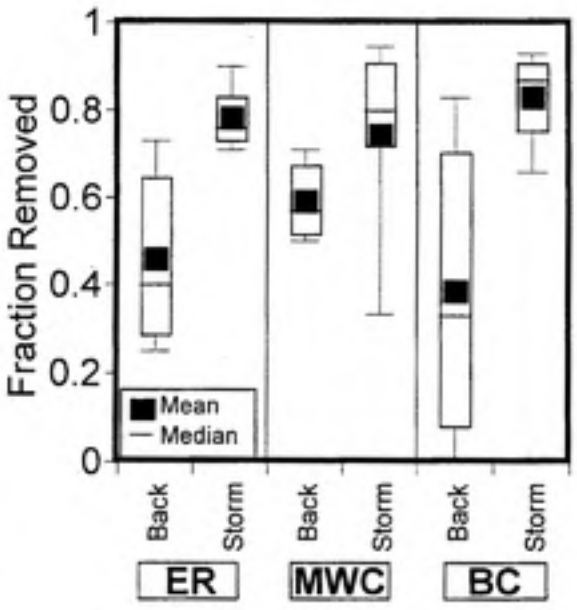


Figure 9: Fraction Removed of TSS by Centrifugation

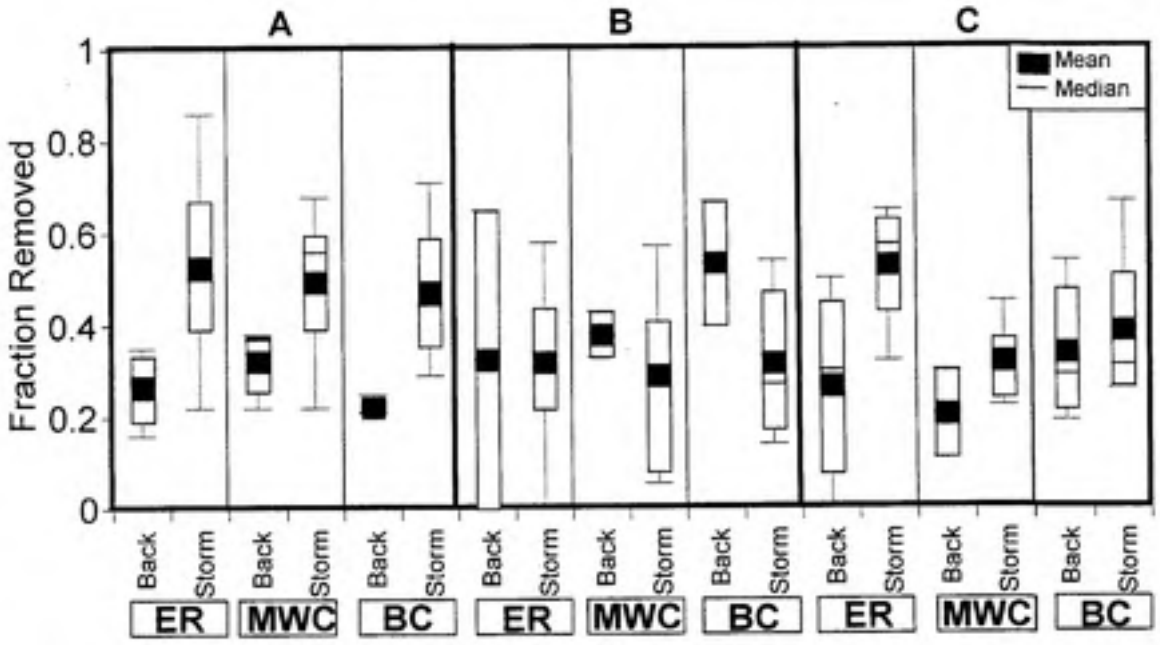


Figure 10: Fraction Removed of Fecal Coliforms (A), *E.* coli (B), and Enterococci (C) by Centrigugation

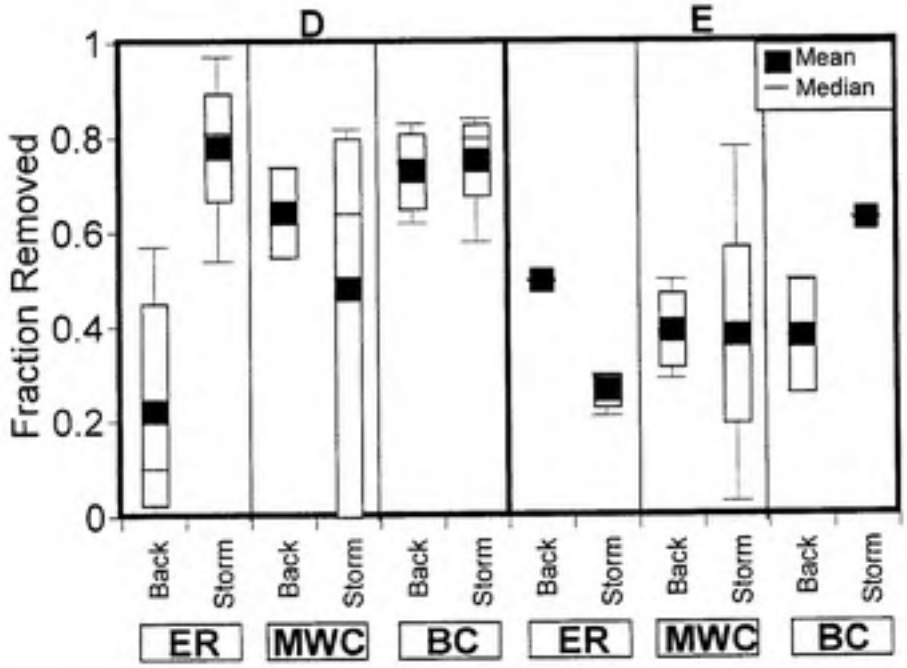


Figure 11: Fraction Removed of *Cl. perfringens* Spores (D) and Total Coliphage (E) by Centrifugation

Background and storm samples are characterized by differences in particle size distributions and, in many cases, differences in the fractions of microbes removed via centrifugation. In order to explore a possible link between these two variables, the fraction of microbes removed is plotted as a function of particle concentration for each organism. In most cases, little evidence of a relationship emerges. The strongest indication of a linear relationship is exhibited by fecal coliforms, although the correlation is still somewhat weak (Figure 12). In addition, graphical analysis of microbial concentration and microbial fraction removed vs. TOC, pH, and temperature did not indicate any relationships (Appendix 2).

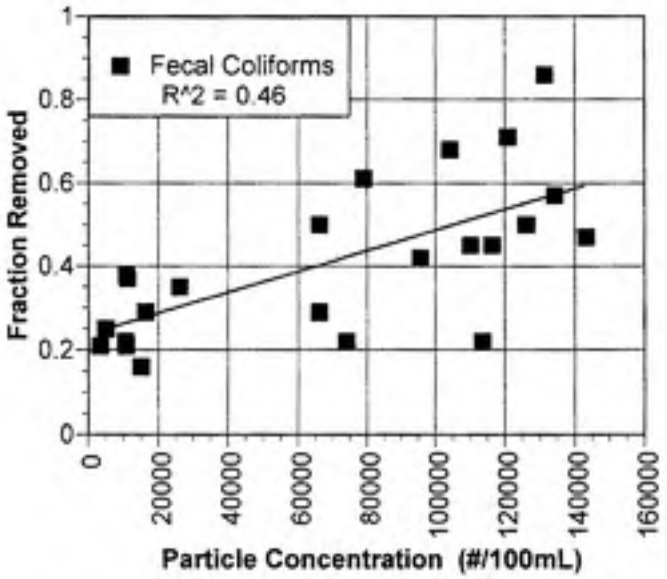


Figure 12: Particle Concentration vs. Fecal Coliform Fraction Removed

4.1.2 Sedimentation Simulations

Simple, but illustrative, simulations of microbial removal via sedimentation were undertaken. The mean size distribution of heavier particles in stormwater (derived from

the difference between raw and centrifuged storm particle size distributions as shown in Figure 8) was separated into 10 μm bins (e.g. 5 to 15 μm) and the percent of total surface area in each size bin was calculated (Figure 13). The mean fraction of each organism removed at each sampling site was used for calculations. In the simulations, microbial partitioning across the particle size distribution was assumed to be proportional to surface area such that if 20% of total particle surface area resided in the 15 to 25 μm size range and 40% of a particular microbe is associated with inorganic/denser particles, then 8% (0.2 x 0.4) of the total number of attached organisms are attributed to particles in this size range. Particles are assumed to have a density of 2.65 g/cm^3 and to settle according to Stokes' Law. In a hypothetical settling basin, particles and associated microbes are assumed to be removed from the water column when particles have settled 2.5 m, a typical design depth of a wet detention basin (NCDENR, 1999).

Microbial partitioning will have a distinct impact on the effectiveness of a wet detention basin in removing each type of microbial organism (Figure 14). Under the assumptions made, fecal coliforms and *Cl. perfringens* spores tend to be removed to a greater extent than *E. coli*. Most microbial removal takes place in approximately the first 8 hours of treatment. This is far below the typical 2 day detention time of detention basins (NCDENR, 1999). These calculations suggest that wet detention basins can remove between 25 and 60% of microbial contaminants, depending on the organism.

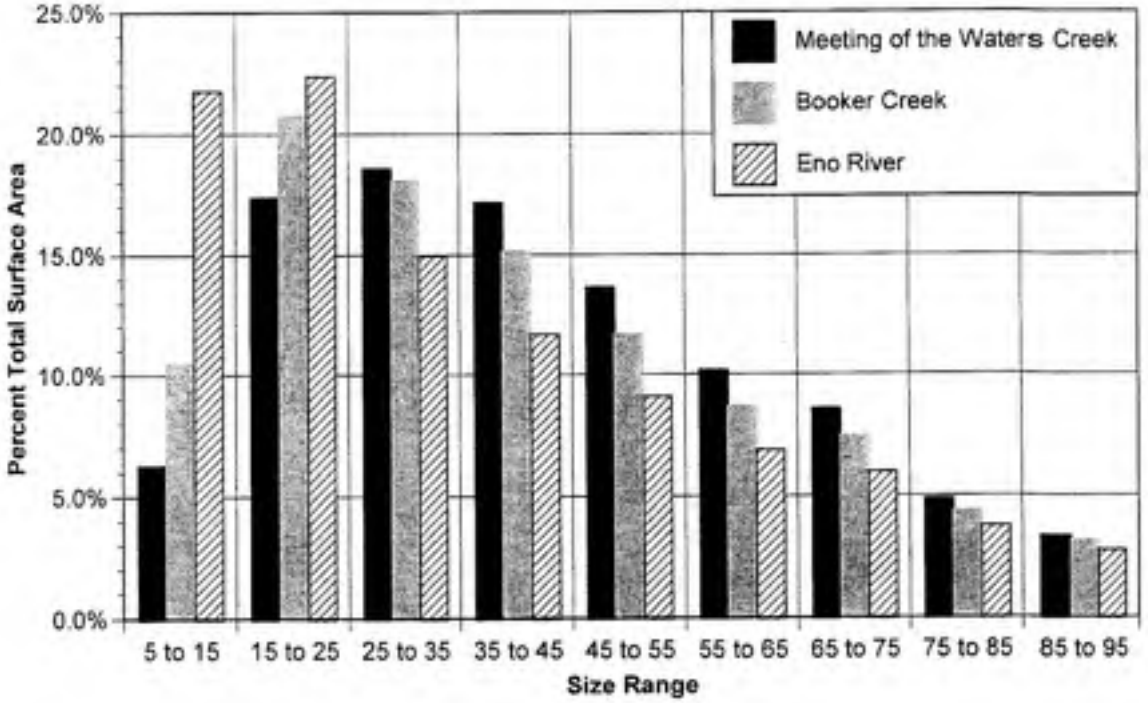


Figure 13: Distribution of Surface Area in Stormwater Samples

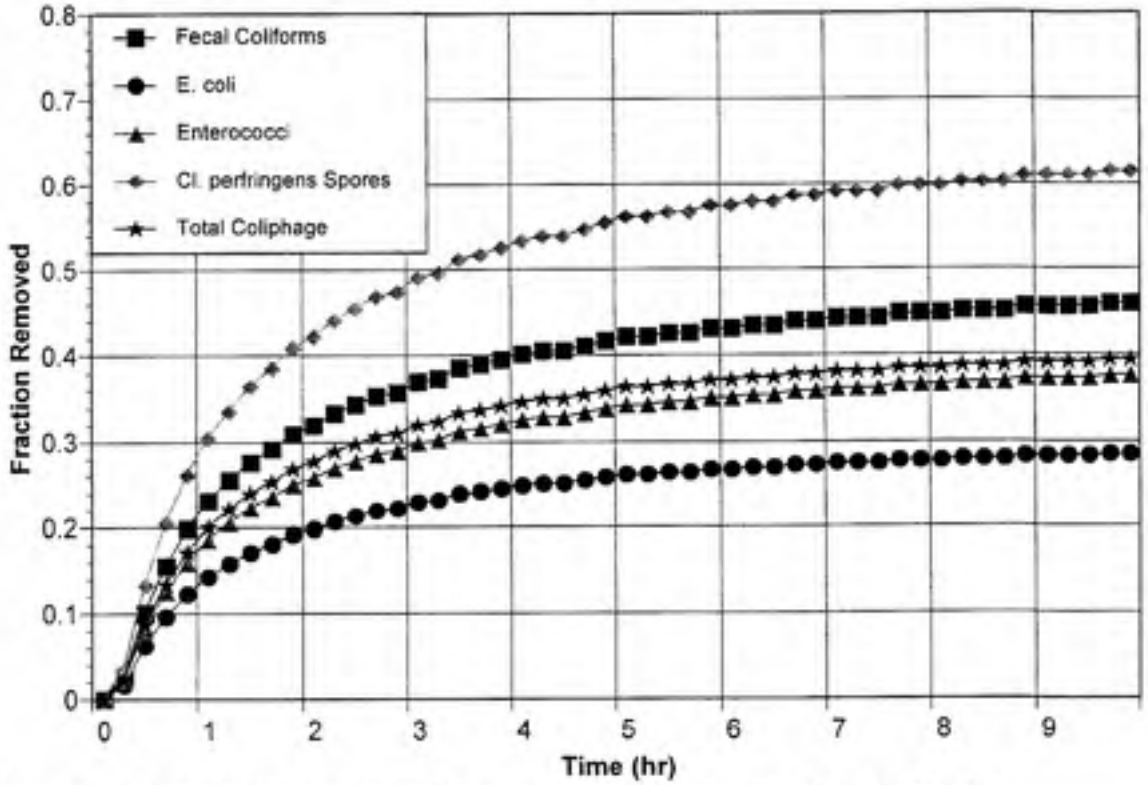


Figure 14: Simulated Microbial Removal in a Stormwater Dention Basin

4.2 Intra-Storm Sampling

4.2.1 Intra-Storm Sampling Results

Intra-storm sampling was undertaken during one storm at Meeting of the Waters Creek (May 1-2, 2004) and Eno River (May 2-3, 2004). Samples were collected throughout the storm events in order to characterize behavior from the point at which flows began to increase, through peak flows, and as flows declined to background levels. Initial samples were important in determining if high contaminant concentrations occurred in the first flush, whereas the timing of peak concentrations was also interesting in terms of estimating contaminant loading. Sampling as the flow declined was also important in exploring how contaminant concentrations vary relative to peak flow.

Due to the difficulty in predicting the onset of a storm event, the initial pre-storm conditions are assumed to be similar to mean background conditions. It is also assumed that conditions return to pre-storm (i.e. average background) concentrations after the storm event. At Meeting of the Waters Creek, typical storm events result in 24-hour hydrographs. Hydrographs at Eno River tend to last 24 to 48 hours, but is assumed to be 24 hours for the sampled storm due to the relatively short rainfall duration. At both sites, the stream flow did not return all the way to background flow levels before another rain event occurred. Thus, the flow for a small portion of each hydrograph was extrapolated in order to obtain 24-hour hydrographs that would result from typical single storm events. The 24-hour hydrographs sampled at Meeting of the Waters Creek and Eno River are shown with the time of each sample in Appendix 3.

Most Probable Numbers (MPNs) and mean values for the sampling events are shown in Tables 4 and 5. Measurements with confidence intervals representative of

analytical uncertainty are presented in Appendix 4, as are time and flow data for each sample.

Table 4: Meeting of the Waters Intra-storm Data

	Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringen Spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration* (#/100mL)	TSS (mg/L)	Settled TOC** (mg/L)
Background								
Raw	4,504	208	206	32	15	10,342	5	2.9
Centrifuged	3,037	324	182	18	9	2,893	2	3.0
Sample 1 (0:45)								
Raw	84,392	2,194	4,249	1,100	6	132,683	638	7.6
Centrifuged	66,295	804	1,281	150	8	23,182	26	8.7
Sample 2 (2:30)								
Raw	66,295	1,929	3,915	>1,100***	4	132,643	462	3.6
Centrifuged	25,515	866	1,613	150	10	18,630	62	3.4
Sample 3 (3:45)								
Raw	22,944	2,083	1,781	460	10	122,409	229	3.5
Centrifuged	5,865	1,101	921	93	6	18,385	19	2.9
Sample 4 (5:00)								
Raw	28,174	2,522	5,727	460	38	125,774	115	4.0
Centrifuged	12,403	1,939	3,081	93	37	19,133	31	3.8
Sample 5 (9:00)								
Raw	17,171	1,989	6,710	460	48	130,622	136	4.5
Centrifuged	23,446	2,996	5,417	43	39	11,780	32	4.2
Sample 6 (17:00)								
Raw	13,596	1,622	4,586	460	24	88,500	43	4.3
Centrifuged	7,750	921	2,978	150	24	9,697	24	4.3

Note: Times (hh:mm) indicate time of collection after onset of storm

* Measurable size range: 5 µm to 100 µm

** Samples remain in the autosampler for some period prior to analysis, potentially allowing organic particles to settle out

*** Missed dilution

Table 5: Eno River Intra-storm Data

	Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringen Spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration* (#/100mL)	TSS (mg/L)	Settled TOC** (mg/L)
Background								
Raw	893	63	45	53	7	18,664	6	3.5
Centrifuged	651	66	36	45	10	4,514	3	3.4
Sample 1 (1:30)								
Raw	24,325	4,061	20,844	460	29	130,895	176	7.2
Centrifuged	3,439	1,714	9,500	15	23	6,884	17	5.5
Sample 2 (4:30)								
Raw	19,835	4,477	17,770	240	27	126,836	130	7.5
Centrifuged	15,744	3,443	28,902	460	49	9,148	32	7.5
Sample 3 (7:00)								
Raw	19,359	4,202	26,795	240	65	118,928	154	8.0
Centrifuged	8,234	2,537	14,246	240	91	10,136	23	7.8
Sample 4 (8:15)								
Raw	11,320	2,666	11,069	460	83	113,854	83	7.9
Centrifuged	4,642	1,231	8,556	460	81	14,717	19	7.5
Sample 5 (10:00)								
Raw	8,712	1,539	8,622	1,100	61	87,942	79	7.2
Centrifuged	5,117	1,168	6,639	93	64	24,836	15	7.2

Note: Times (hh:mm) indicate time of collection after onset of storm

* Measurable size range: 5 μm to 100 μm

** Samples remain in the autosampler for some period prior to analysis, potentially allowing organic particles to settle out

Results are presented in a manner that explores the changes in concentration of particle number, TSS, and microbial concentration throughout individual storm events. The stacked columns on the primary axis indicate concentrations in the raw sample (total height of each column) and concentration in the centrifuged sample (remaining in suspension), with the difference representing the fraction removed (cross hatched area). Values on top of the columns indicate the fraction of each constituent removed via centrifugation. A single asterisk (*) indicates that the concentration in the centrifuged sample exceeded that in the raw sample. A double asterisk (**) indicates a missed dilution in a microbial sample. The flow is shown as a line, with units of cubic meters per second (cms).

At both sites, particle concentrations are consistently high throughout the storm event (Figures 15 and 16). The fraction of particles removed is also high at both sites, with a low value of 0.72 at Eno River. Removal is relatively consistent (varying from 0.83 to 0.91) at Meeting of the Waters, whereas removal decreases slightly throughout the hydrograph at Eno River (0.95 to 0.72). The highest particle concentrations at both sites occur before the peak flow, perhaps an indication of a first flush.

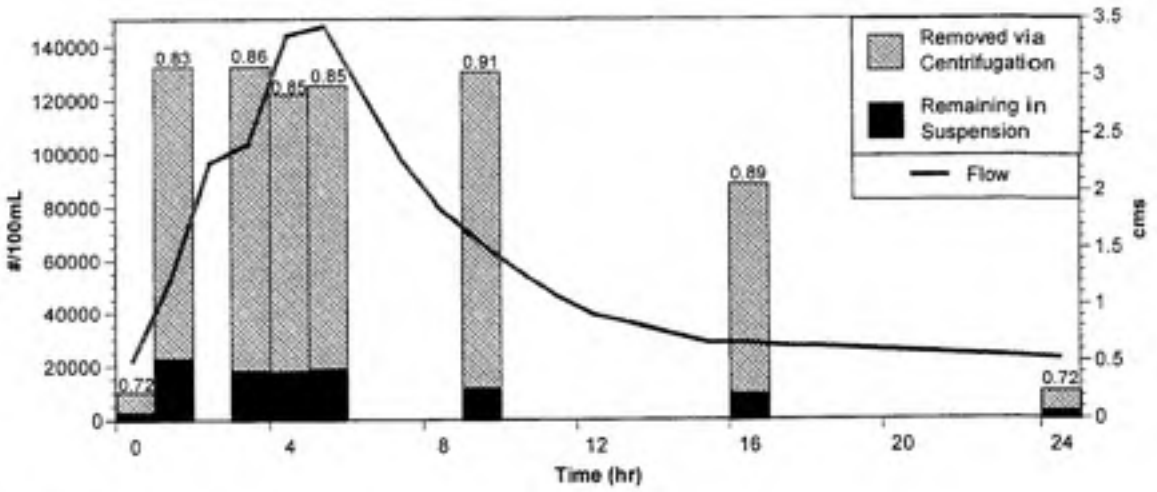


Figure 15: Particle Behavior and Flow at Meeting of the Waters Creek

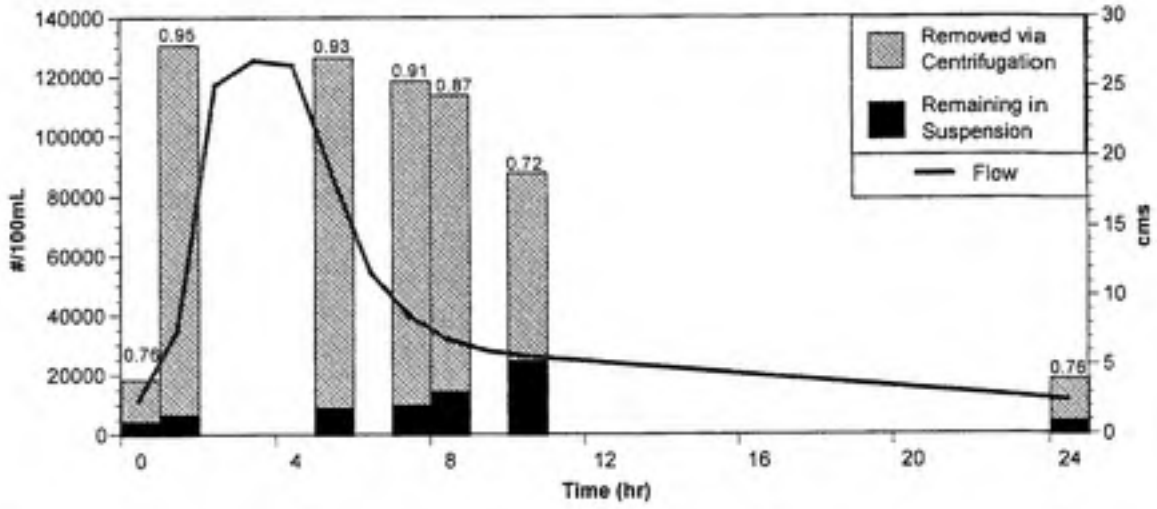


Figure 16: Particle Behavior and Flow at Eno River

Data on TSS also supports the notion of a first flush (Figures 18 and 19). At both sites, concentrations are highest in the first storm sample on the upswing of the hydrograph and generally decrease throughout the hydrograph. At Meeting of the Waters Creek, higher TSS concentrations generally correspond to higher fractions removed.

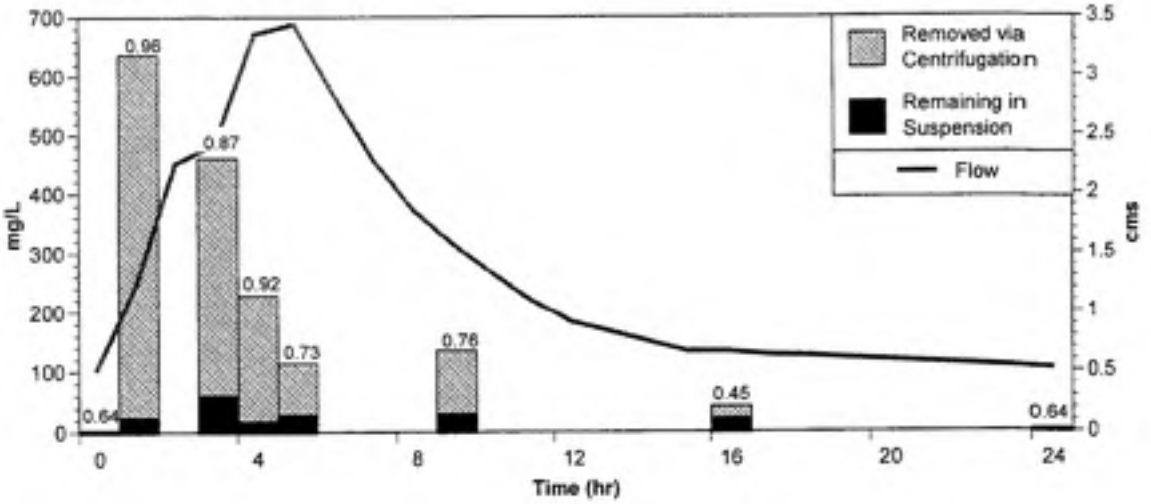


Figure 17: TSS Behavior and Flow at Meeting of the Waters Creek

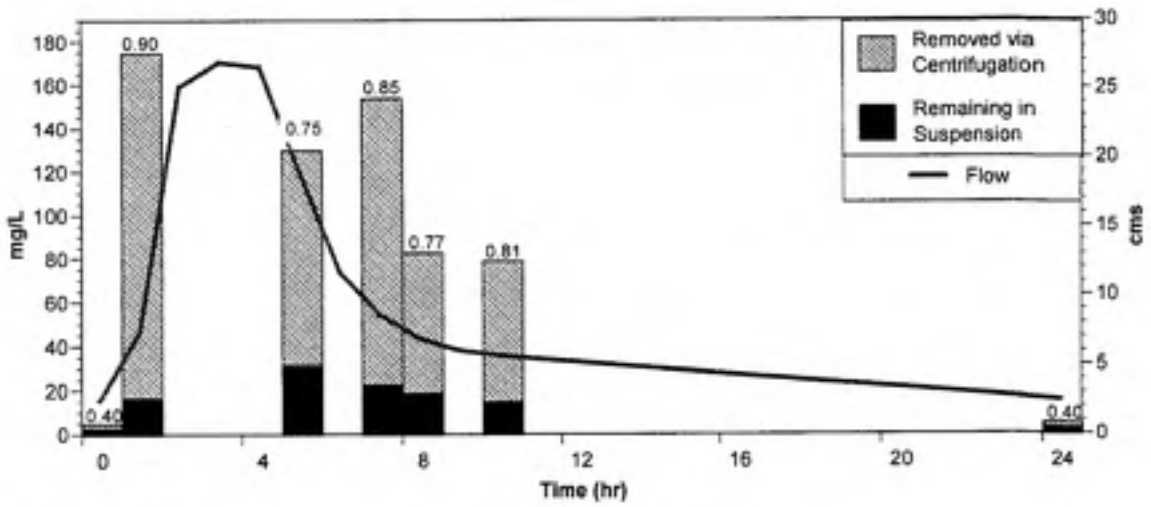
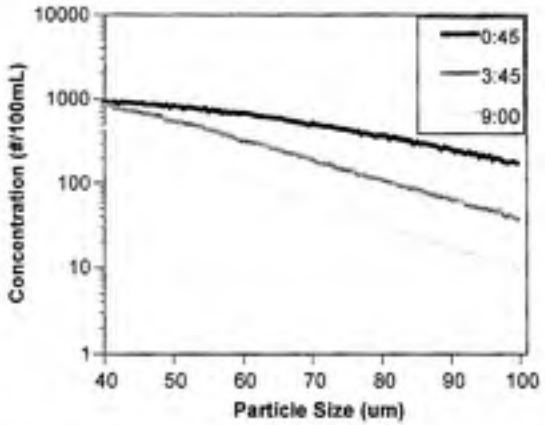
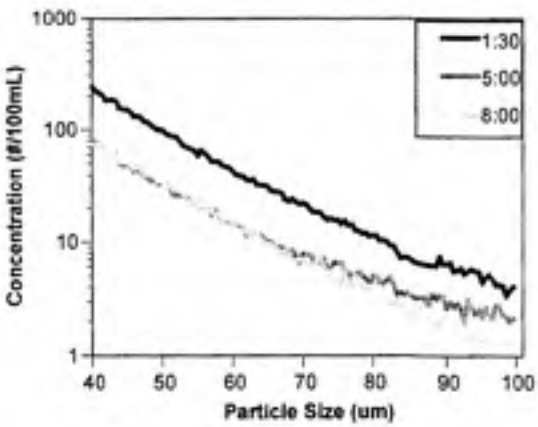


Figure 18: TSS Behavior and Flow at Eno River

The particle and TSS concentrations suggest that heavier and/or larger particles are mobilized by stormwater, particularly in the early stages of a storm. This is reinforced in Figures 19 and 20, which show that the concentrations of larger particles decrease throughout the storm events. These larger particles may be responsible for the

initial increase in particle and TSS concentration. As the storm progresses, the concentration of larger particles declines while the concentration of smaller particles increases. This results in the observed high particle concentrations and low TSS concentrations.





Note: 1st, 2nd, and 3rd samples at each site are denoted by the sampling time (hours:minutes) after flows began to increase.

Figures 19 and 20: Particle Size Distribution of 1st, 3rd, and 5th Samples at Meeting of the Waters Creek and Eno River

Fecal coliforms demonstrate behavior similar to TSS and therefore may also be affected by the first flush (Figures 21 and 22). Both contaminants follow the same general trend throughout the hydrograph. At both sites, the first storm sample has the highest concentration and the concentration of subsequent samples show a downward trend. There may also be a potential link between removal of TSS and fecal coliforms via centrifugation. At Eno River, the greatest fraction removed occurs at the same point for both TSS and fecal coliforms. Overall, the nature of the partitioning relationship is not clear. While there are concentration trends, there was no clear trend between the

fractions of each contaminant removed and thus more conclusive statements regarding the nature of a partitioning relationship will have to wait for additional data.

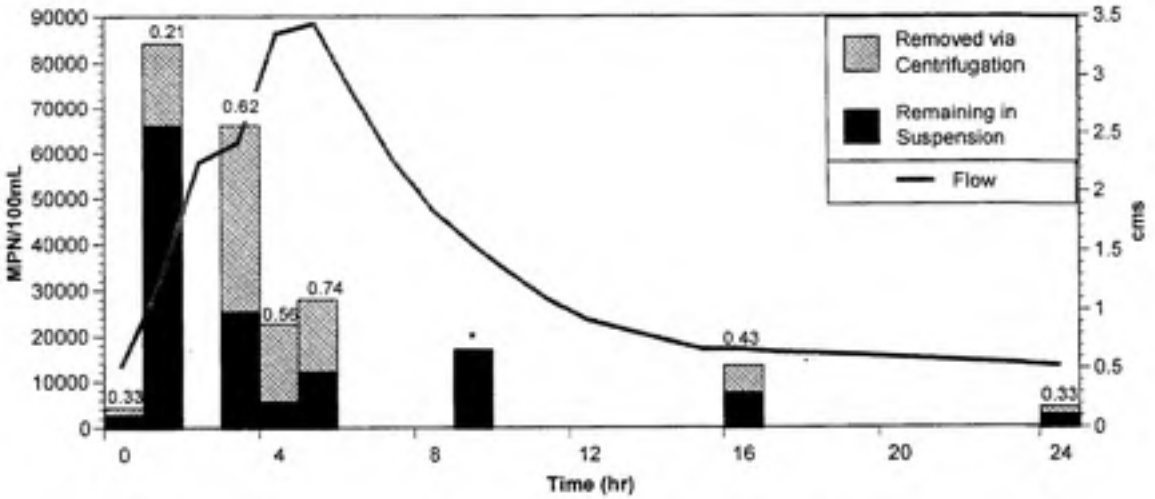


Figure 21: Fecal Coliform Behavior and Flow at Meeting of the Waters Creek

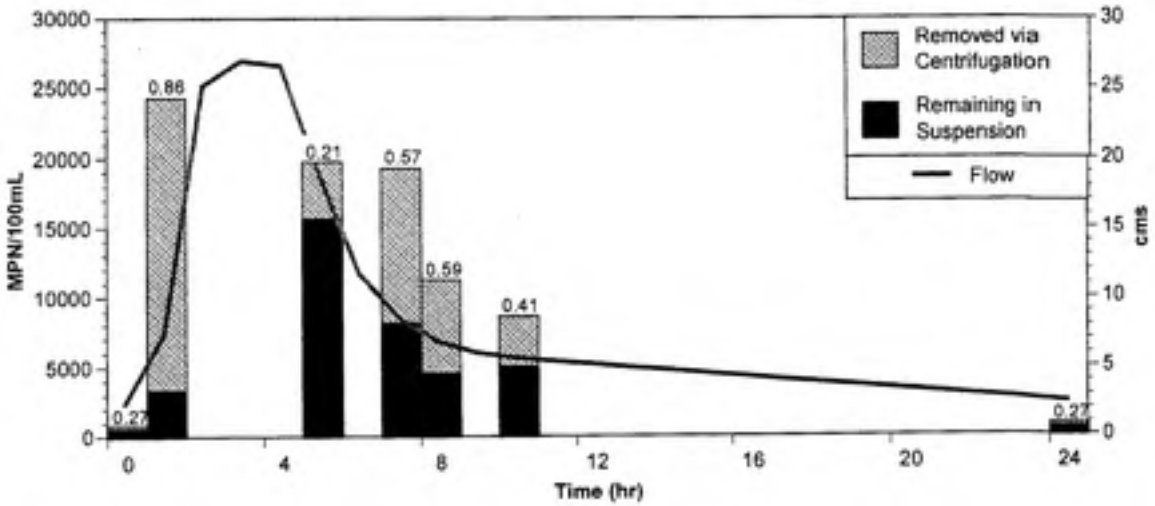


Figure 22: Fecal Coliform Behavior and Flow at Eno River

The concentrations and fractions removed of *E. coli* behave similarly at Meeting of the Waters Creek and Eno River (Figures 23 and 24). *E. coli* concentrations tend to

rise and fall with flow. With the exception of the first storm sample at Meeting of the Waters, concentration increases until peak flow, and then decreases. The highest concentrations tend to occur close to the peak flow. At both sites, the highest concentration corresponds to the lowest fraction of *E. coli* removed. This trend is consistent with grab sample data, which indicated that the fraction removed decreased during storm events. If the partitioning of *E. coli* was dependent upon the presence of certain particle sizes (i.e. larger or small), maximum removal would not occur at peak flow because there tends to be more large particles before peak flow and more small particles after peak flow. In addition, the partitioning behavior of *E. coli* does not appear to be related to *E. coli* concentrations. There are no apparent relationships between concentration and fraction removed in either the grab samples or the intra-storm samples. The partitioning behavior of *E. coli* does tend to change from background to storm conditions and throughout the hydrograph, but no clear partitioning relationship emerges.

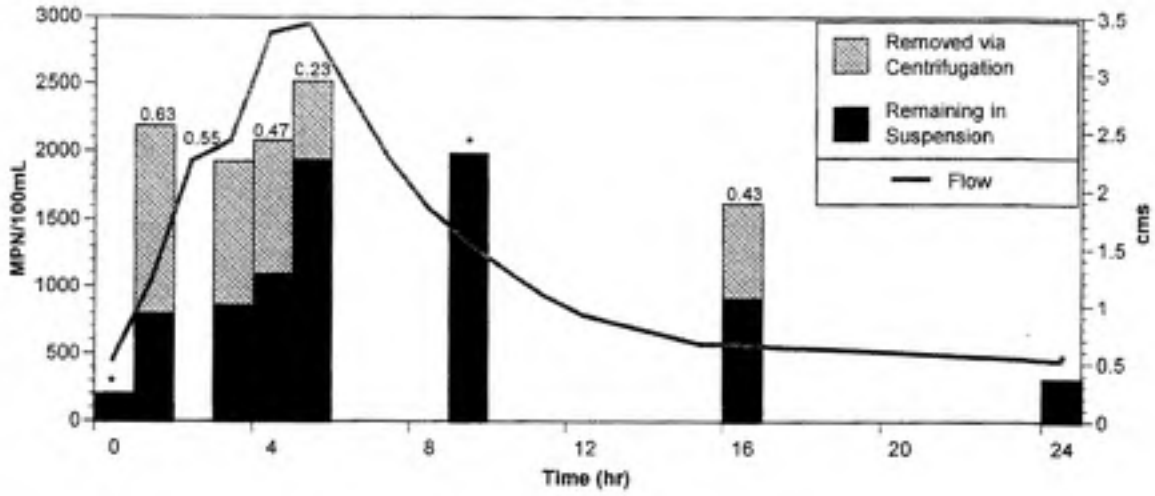


Figure 23: *E. coli* and Flow at Meeting of the Waters Creek

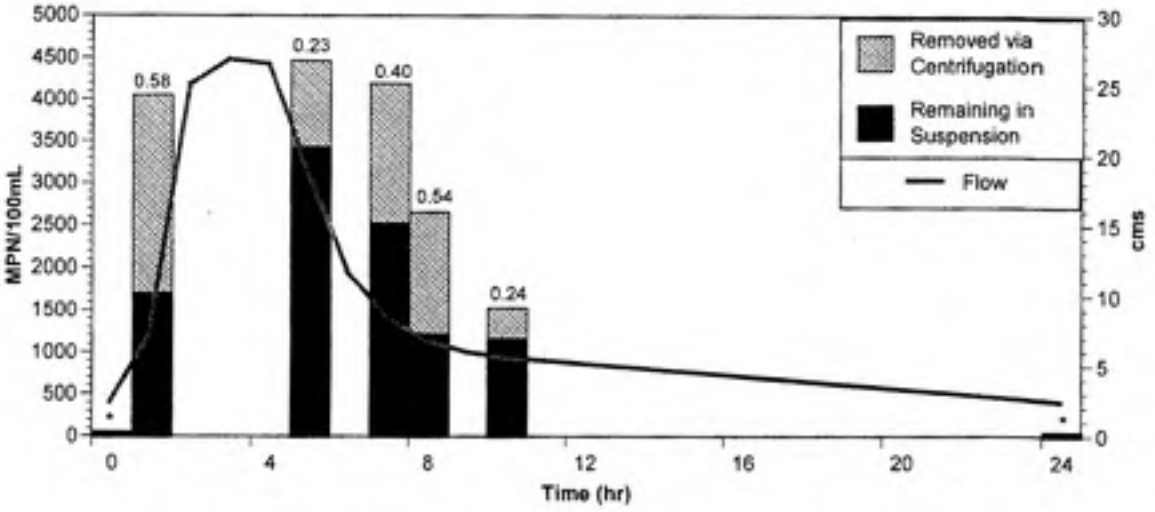


Figure 24: *E. coli* and Flow at Eno River

Enterococci concentrations are highest after peak flow at both sampling locations (Figures 25 and 26), a departure from the behavior exhibited by fecal coliforms and *E. coli*. It is also interesting to note that the fraction of enterococci removed is greater in the storm samples than in the background samples. At Meeting of the Waters, the highest concentration corresponds to the lowest fraction of enterococci removed throughout the

storm samples, but this behavior was not observed at Eno River. Previous research (Davies and Bavor, 2000) has indicated that enterococci may preferentially associate with small particles (defined as <2μm in their study). Enterococci may be partitioning to small particles during the storm events, resulting in high concentrations after peak flows when a higher fraction of total particle concentrations are small.

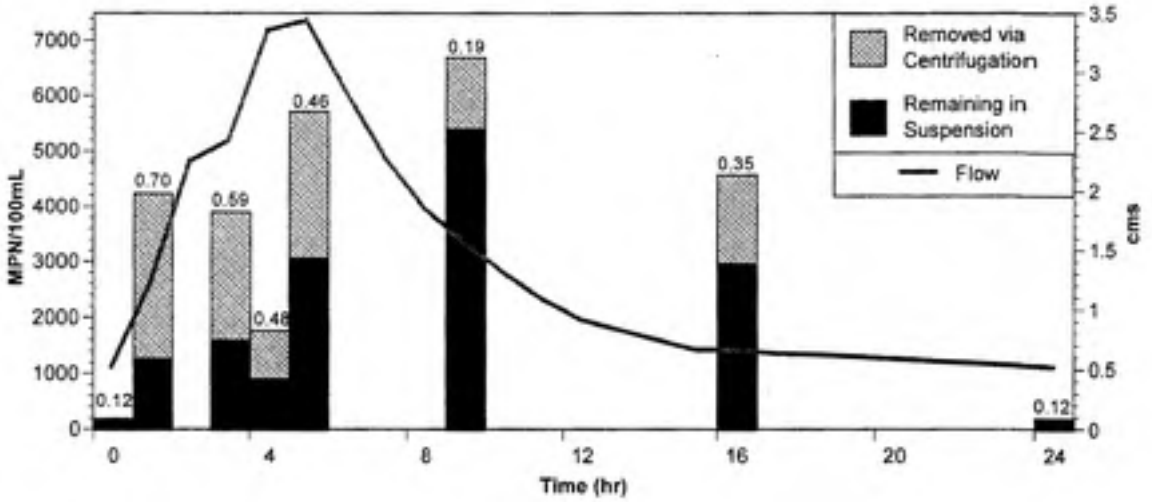


Figure 25: Enterococci Behavior and Flow at Meeting of the Waters Creek

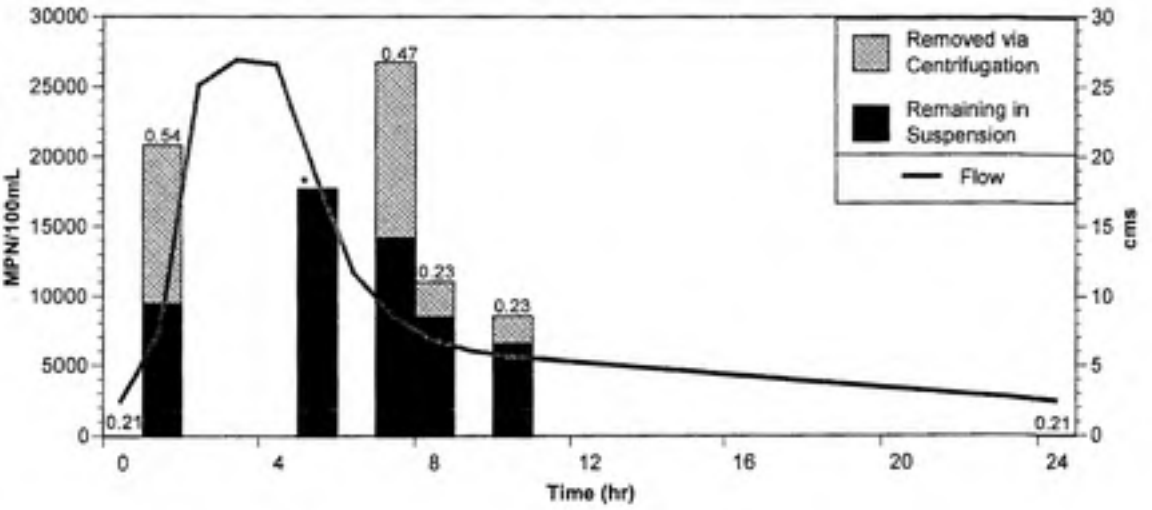


Figure 26: Enterococci Behavior and Flow at Eno River

Cl. perfringens spore concentrations demonstrated the most variability between sites (Figures 27 and 28). At Meeting of the Waters, concentrations are highest on the upswing of the hydrograph. The fraction removed is consistently high, with a low value of 0.67. Removal efficiencies for TSS and *Cl. perfringens* spores were very similar. For five of the six samples, the fraction of *Cl. perfringens* spores removed is within 0.05 of the corresponding fraction of particles removed. This may indicate a strong partitioning relationship. Behavior at Eno River is more variable. *Cl. perfringens* spore concentrations are highest on the upswing of the hydrograph. The fraction removed is dichotomous; values are either greater than 0.90 or less than 0.16.

Because of the variability of *Cl. perfringens* concentrations and removal efficiencies, it is difficult to identify trends. While the fraction of *Cl. perfringens* spores removed via centrifugation is generally high (greater than 0.67), there are some storm values where removal was minimal. However, the partitioning relationship appears to be strong for many of the individual data points throughout the hydrograph and this is consistent with results from the grab samples.

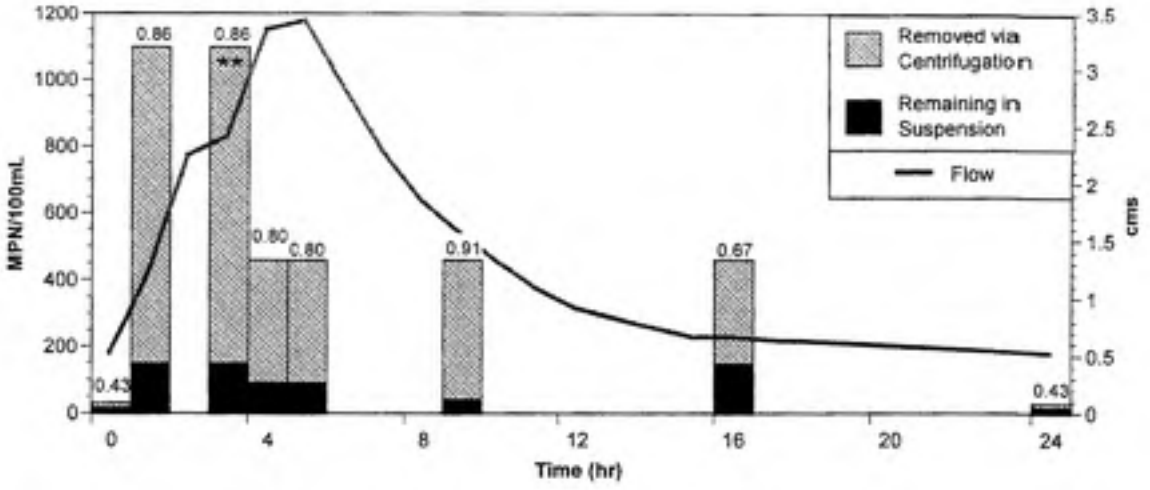


Figure 27: *Cl. perfringens* Spores and Flow at Meeting of the Waters Creek

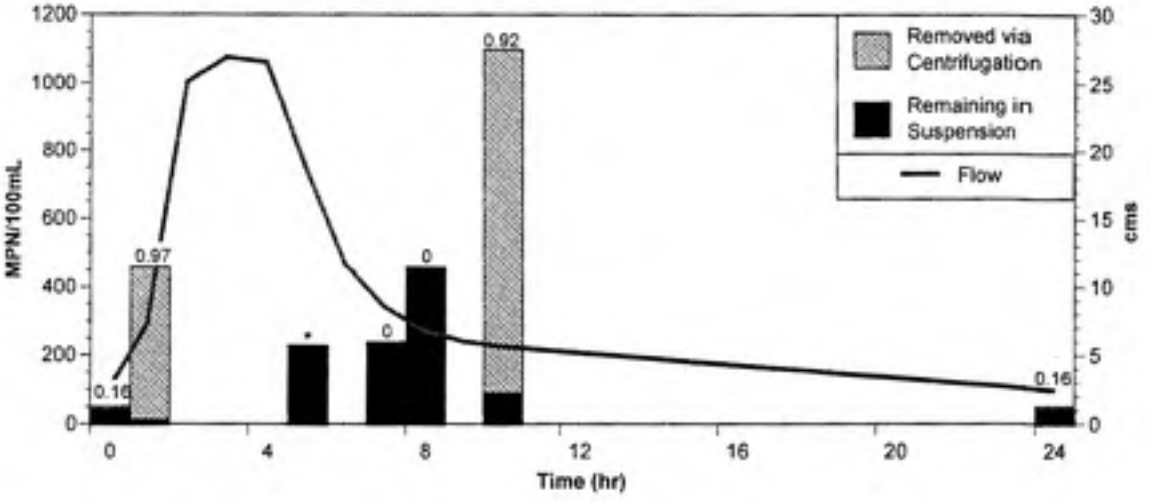


Figure 28: *Cl. perfringens* Spores Behavior and Flow at Eno River

Total coliphage demonstrates the weakest partitioning relationship in grab samples and throughout the hydrograph (Figures 29 and 30). At both sites, total coliphage concentration is at its peak after peak stream flow. At Meeting of the Waters, a larger fraction of organisms appears to be associated with particles in the background than in the storm samples. The fraction of total coliphage removed tends to be low

throughout the hydrograph, suggesting that few of these microbes are associated with heavier (i.e. inorganic) particles.

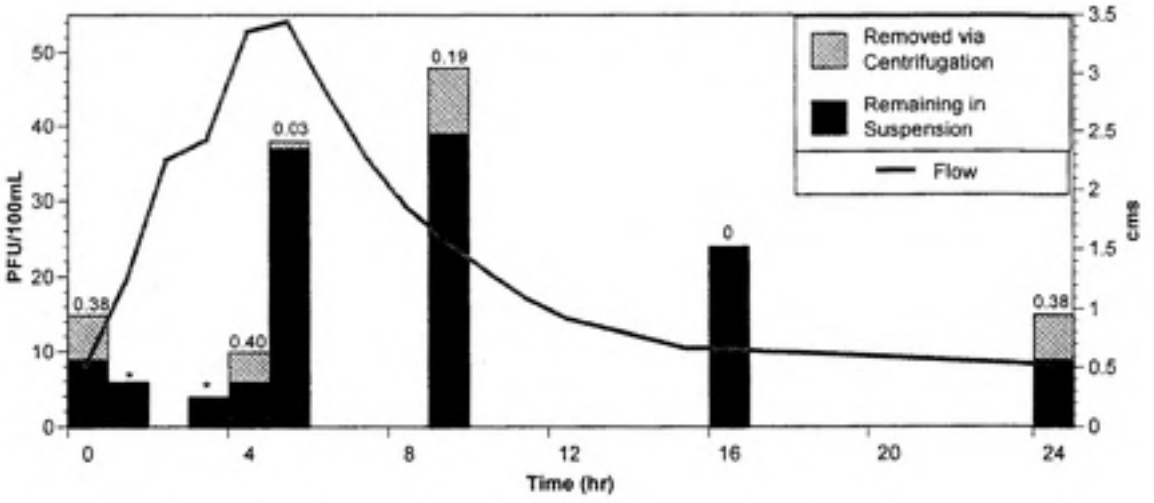


Figure 29: Total Coliphage and Flow at Meeting of the Waters Creek

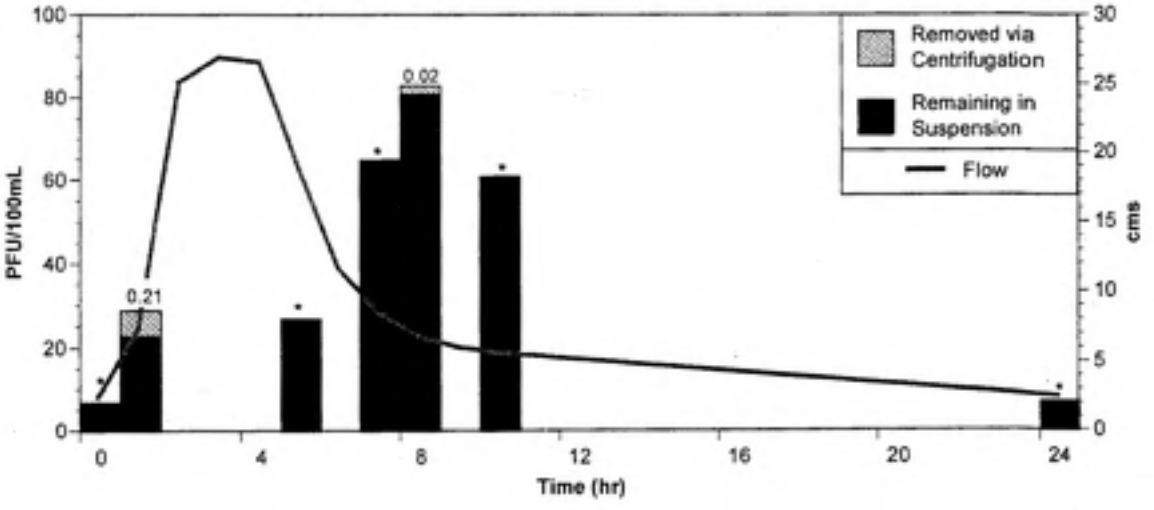


Figure 30: Total Coliphage Behavior and Flow at Eno River

4.2.2 Contaminant Loading

During a storm event, the streams are subject to both higher concentrations of contaminants and higher flows. The increased loading of microbial organisms during the storm events are estimated by interpolating contaminant concentrations between sample points and using available flow data. Data from the hydrograph are used to estimate contaminant concentrations at fifteen minute intervals. Using the average concentration and flow during each fifteen minute interval, incremental loading values are calculated. These incremental loadings are summed over the entire hydrograph to obtain estimates of the total loading from the storm events at each site.

Flow downstream of Meeting of the Waters sampling site is a slight over estimation of the actual flow. The USGS flow values include the discharge from the wastewater plant which is located after the sampling location but before the flow gage. Due to the high increase in stormwater flow during a storm event relative to the wastewater effluent inputs, the effect on loading calculations is assumed to minimal.

The total loading to the water body increases throughout the storm event, as shown by Cumulative Distribution Functions (CDFs) in Figures 31 and 32. Because fecal coliforms tend to have high concentrations earlier in the storm event whereas total coliphage tends to have high concentrations later in the storm event, the loading of fecal coliforms increases much more rapidly than total coliphage loading at both sites.

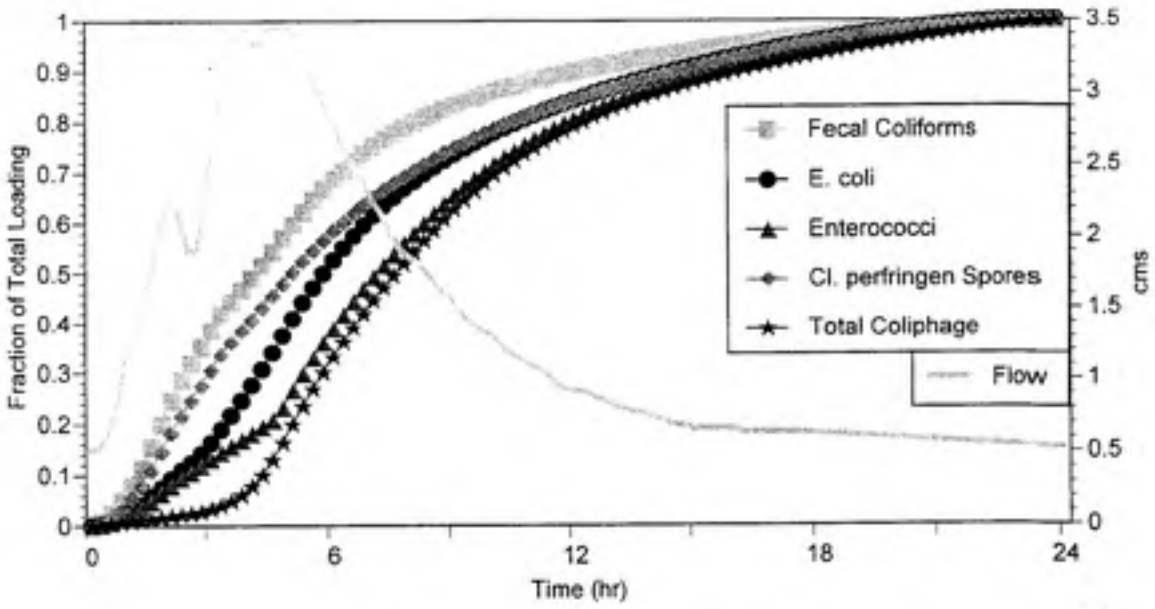


Figure 31: Contaminant CDF and Flow at Meeting of the Waters

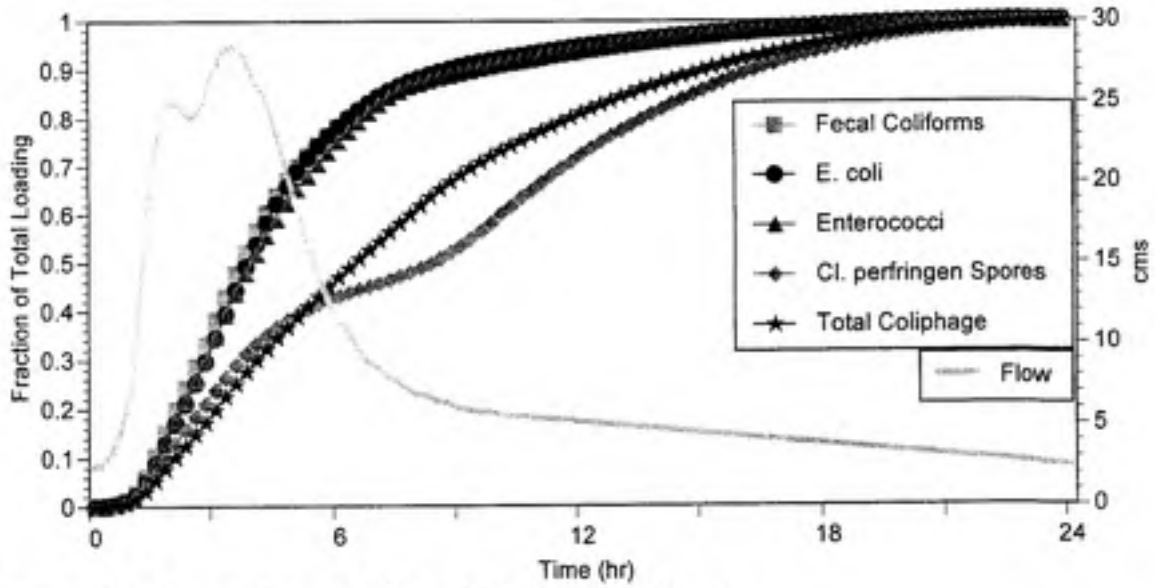


Figure 32: Contaminant CDF and Flow at Eno River

The loading that results from a storm event is much higher than background loading, as shown in Table 6. The storm contaminant loading can be equivalent to the contaminant loading of many days under background conditions.

Table 6: 24-hour Contaminant Loading under Storm and Background Conditions

	24-hour Storm Loadings		24-hour Background Loadings		Equivalent Storm Loading in Background Days
Meeting of the Waters Creek					
Fecal Coliforms	3.09E+10	CFU	1.98E+09	CFU	16
E. coli	2.21E+09	CFU	9.16E+07	CFU	24
Enterococci	5.13E+09	CFU	9.09E+07	CFU	56
Cl. perfringen Spores	5.97E+08	CFU	1.40E+07	CFU	43
Total Coliphage	3.22E+07	PFU	6.61E+06	PFU	5
TSS	1.99E+04	kg	2.25E+02	kg	89
Eno River					
Fecal Coliforms	1.12E+11	CFU	1.68E+09	CFU	67
E. coli	2.22E+10	CFU	1.26E+08	CFU	176
Enterococci	1.06E+11	CFU	9.09E+07	CFU	1166
Cl. perfringens Spores	3.07E+09	CFU	1.34E+07	CFU	229
Total Coliphage	2.65E+08	PFU	1.12E+07	PFU	24
TSS	8.11E+04	kg	1.12E+03	kg	73

An analysis was undertaken to estimate the effectiveness of a simple detention basin in treating stormwater runoff. North Carolina stormwater regulations require an 85% TSS reduction in the first one inch of rainfall. The rainfall at and around Meeting of the Waters Creek ws estimated to be 0.74 inches, and it was therefore assumed that all runoff would be treated in a stormwater treatment scenario. The rainfall at and around Eno River is estimated was 1.33 inches, and it was therefore assumed that the first 75% of the flow would be treated in a stormwater treatment scenario. For treated stormwater, the particle distribution of the heavier particles (i.e. those removed by centrifugation) was extrapolated throughout the hydrograph. Particles were assumed to enter the hypothetical detention basin at 15 minute intervals over a 24 hour period, and the water was held for 24 hours from the onset of treatment. These particles settled according to Stokes' Law assuming a particle density of 2.65 g/cm^3 and a settling distance of 2.5 m. Microbial

removal was interpolated from the fraction of each contaminate removed for each sampling point over the hydrograph.

Table 7 summarizes the microbial contaminant loading under conditions in which runoff does or does not receive primary treatment (i.e. sedimentation) through the use of detention basins. All of the stormwater at Meeting of the Waters Creek is assumed to receive treatment, but only the first 11 hours of storm flow at Eno River would receive treatment. Because some of the runoff would not be treated, reductions in contaminant loadings tend to be lower at Eno River.

Table 7: Contaminant Loadings with Stormwater Treatment

	Storm Loadings with Stormwater Treatment		Storm Loadings without Stormwater Treatment		Reduction in Contaminant Loading Due to Treatment
Meeting of the Waters Creek					
Fecal Coliforms	1.47E+10	CFU	3.09E+10	CFU	52%
E. coli	1.40E+09	CFU	2.21E+09	CFU	37%
Enterococci	3.28E+09	CFU	5.13E+09	CFU	36%
Cl. perfringen Spores	1.15E+08	CFU	5.97E+08	CFU	81%
Total Coliphage	2.79E+07	PFU	3.22E+07	PFU	13%
Eno River					
Fecal Coliforms	5.91E+10	CFU	1.12E+11	CFU	47%
E. coli	1.41E+10	CFU	2.22E+10	CFU	36%
Enterococci	5.85E+10	CFU	1.06E+11	CFU	45%
Cl. perfringen Spores	1.87E+09	CFU	3.07E+09	CFU	39%
Total Coliphage	2.43E+08	PFU	2.65E+08	PFU	8%

The overall reductions in contaminant loadings can be used to determine the effectiveness of detention basins in stormwater treatment, while contaminant removal over time can further illustrate particle-microbial settling behavior. The fraction of total loading removed for each microbial contaminant is shown in Figures 33 and 34. At Meeting of the Waters Creek, *Cl. perfringens* spores and fecal coliforms experience the

greatest removal and total coliphage exhibits the least. The high removal of *Cl. perfringens* spores is due to the high particle association and the occurrence of peak concentrations in the first portion of runoff. Fecal coliform removal is also high because fecal coliforms demonstrate a relatively strong particle association and concentrations are highest before peak flows. The opposite is true for total coliphage. The removal rate is consistently low and the highest concentrations occur as flows are declining, resulting in the lowest fraction of microbial loading removed.

The behavior of *Cl. perfringens* spores in not consistent for the two sites. *Cl. perfringens* spores are removed less effectively at the Eno River than at Meeting of the Waters. The plateau in *Cl. perfringens* spore removal is due to the measured removals of 0 in two of the hydrograph samples (Figure 29). In addition, enterococci are the only contaminant that experiences a higher fractional removal at Eno River than at Meeting of the Waters Creek.

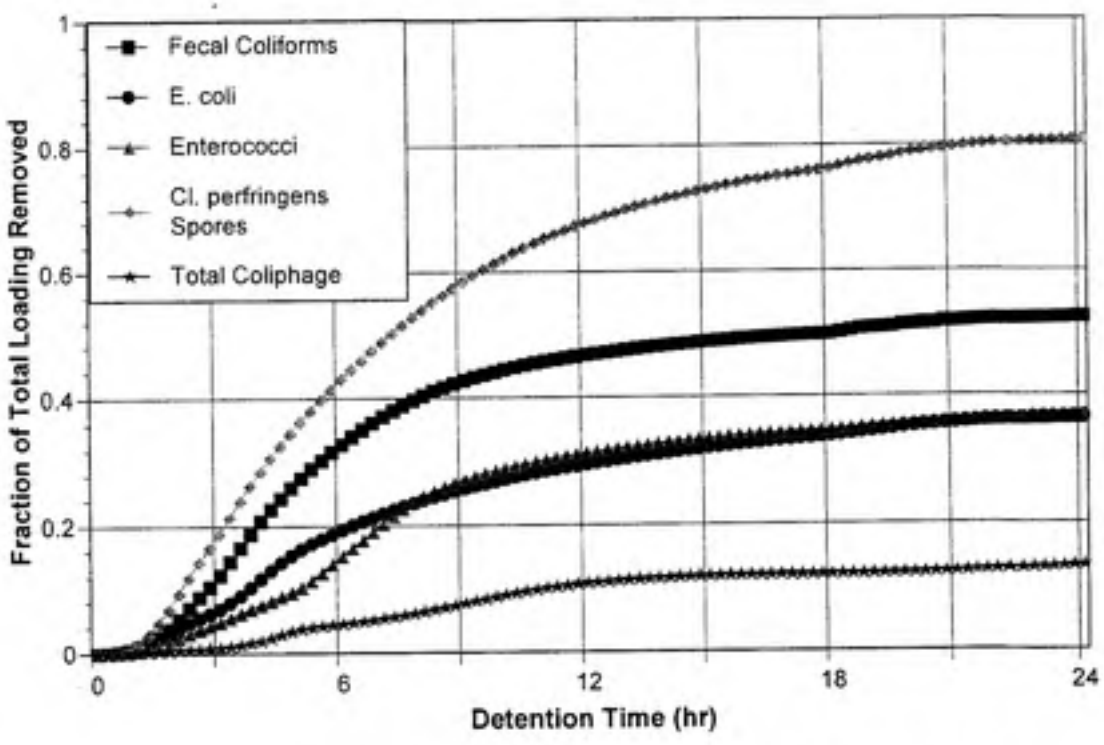


Figure 33: Fraction of Total Loading Removed over Time at Meeting of the Waters Creek

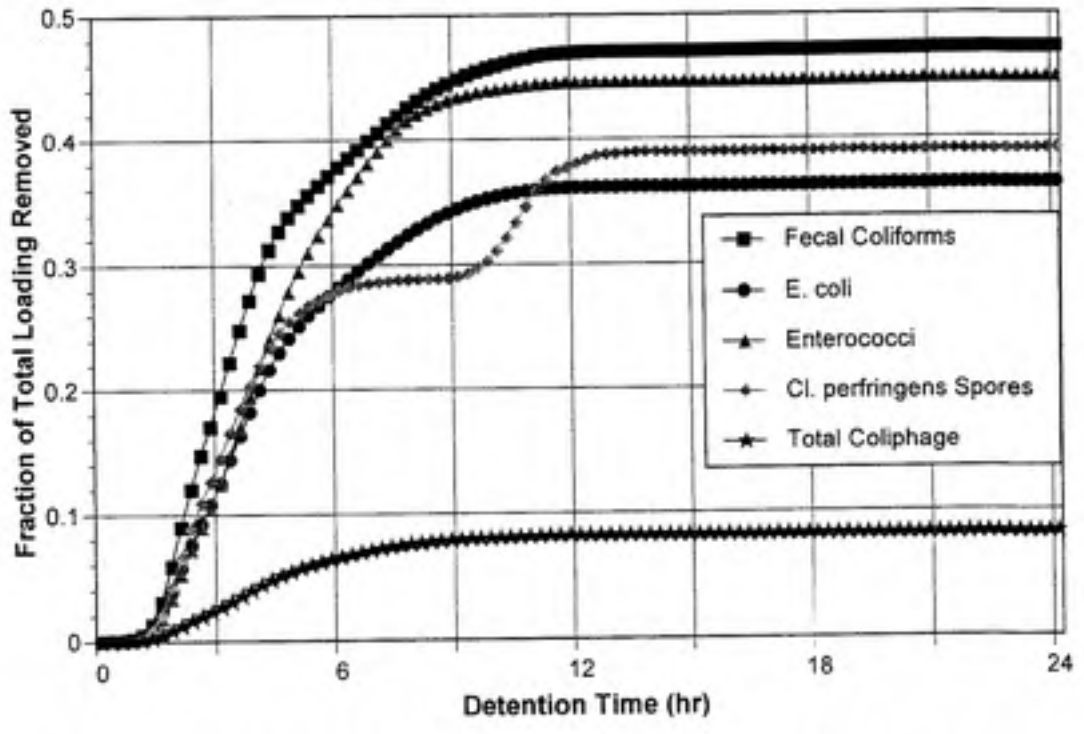


Figure 34: Fraction of Total Loading Removed over Time at Eno River

Table 7 indicates that detention basins are effective in removing substantial loadings of most contaminants investigated, while Figures 33 and 34 demonstrate how these microbial removals are achieved over time. These results can have important implications in stormwater treatment design; the percent reduction of each microbial contaminant can be used to predict the effectiveness of using detention basins in the migitation of microbial contamination that results from stormwater runoff.

Conclusions and Recommendations

5.1 Conclusions

This study has undertaken an initial characterization of the partitioning of several microbial species (fecal coliforms, *E. coli*, enterococci, *Clostridium perfringens* spores, and total coliphage) in urban stormwater runoff by investigating potential links between microbial concentration, particle concentration and different particle size classes. Based on the grab sampling and intra-storm sampling conducted, initial conclusions about microbial concentrations and partitioning have been developed.

Partitioning relationships vary by microbe. In addition, some partitioning relationships differ in background vs. storm conditions and throughout the storm hydrograph. The most notable relationships occur with fecal coliforms, *Cl. perfringens* spores, and total coliphage:

- Fecal coliforms demonstrate the most consistently strong partitioning relationship. Approximately 28% of fecal coliforms in background samples and 58% in storm samples may associate with heavier particles.
- *Cl. perfringens* spores also tend to associate with particles, although results are not as consistent. On average, 61% may associate with heavier particles under storm conditions, but intra-storm removal varied from 0 to 97%.
- Total coliphage removal was consistently low under background and storm conditions. The average removal based on average raw and centrifuged concentrations at the three sampling locations was 13%.

Stormwater runoff is characterized by increased microbial and particle concentrations relative to background concentrations. The particle concentration usually remains consistently high throughout the storm hydrograph, although the occurrence of peak microbial concentration varies by microbe and, in some cases, by sampling site. The behavior of each microbe is as follows:

- Peak concentrations of fecal coliforms tend to occur as flow is increasing. Because TSS also has highest concentrations in the first flush, fecal coliforms may be associated with larger particles that are responsible for the majority of TSS.
- *E. coli* concentrations tend to be highest at peak flows.
- Enterococci and total coliphage concentrations tend to be at a maximum when flow is declining.
- The concentrations of *Cl. perfringens* spores did not demonstrate similar behavior at the two sampling locations.

Peak microbial concentrations and microbial partitioning relationships are essential in determining the effectiveness of sedimentation as a microbial treatment strategy and modeling microbial transport in a natural water body. Stormwater detention basins may effectively remove a substantial portion of microbial contaminant loadings via the settling of microbes attached to particles. Incorporating microbial partitioning into water quality models that serve as the basis for regulatory programs (e.g. NPDES, TMDL) may improve the effectiveness of the models to predict microbial transport. The characterization of microbial behavior in stormwater runoff and the application of the

partitioning relationships in stormwater treatment and policy will have important applications in reducing nonpoint source pollution, thus improving the quality of the receiving waters.

5.2 Recommendations

This study has taken the initial steps in developing particle-microbial relationships in stormwater, but further characterization may be needed. Due to the highly variable nature of microbes in the environment, further characterization should be undertaken by additional intra-storm sampling. With an increased data set, more accurate partitioning relationships could be developed.

These partitioning relationships could be used to determine where stormwater treatment is needed and how effective treatment could be. The partitioning relationships should be incorporated into existing fate and transport models, many of which currently assume all microbes are in the free-phase. These improved models could not only predict how far microbes are transported in a water body more accurately, but could also identify where these organisms eventually come to reside. The relationships could also be used in stormwater treatment design by modeling microbial behavior in a detention basin.

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Appendix 1

Grab sample data are shown for all individual sampling events at all sites. Measured values are shown in bold, while 95% confidence interval are shown above (lower confidence value) and below (upper confidence value). The sampling time relative to peak flow (± hours:minutes) is also shown for Eno River and Meeting of the Waters Creek. It should be noted, however, that the duration of each hydrograph varied with storm event.

Eno River

	Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringens spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background 1								
	1,098	47	24	31	0	15,788	6	4.8
Raw	**1,249**	**65**	**36**	**135**	**2**	**15,788**	**8**	**5.2**
	1,421	90	55	347	7	15,788	10	5.5
	716	39	26	31	0	3,434	5	4.5
Settled	**927**	**56**	**39**	**121**	**1**	**3,434**	**6**	**4.6**
	927	79	59	420	5	3,434	7	4.7
Background 2								
	429	89	68	0	8	14,565	5	2.5
Raw	**495**	**114**	**90**	**3**	**14**	**14,565**	**5**	**2.7**
	571	146	118	12	23	14,565	6	2.8
	355	103	45	0	10	4,066	3	2.4
Settled	**413**	**131**	**63**	**4**	**17**	**4,066**	**3**	**2.6**
	479	165	87	18	27	4,066	4	2.8
Background 3								
	750	3	3	5	1	24,375	2	2.7
Raw	**936**	**10**	**10**	**21**	**4**	**25,638**	**4**	**2.7**
	1,168	40	40	42	10	26,900	5	2.7
	485	3	1	1	6	5,840	0	2.8
Settled	**613**	**10**	**5**	**9**	**11**	**6,042**	**1**	**2.9**
	774	40	36	38	18	6,243	2	3.0
Storm 1 (-6:15)								
	3,160	281	281	42	22	65,648	17	
Raw	**4,098**	**522**	**522**	**240**	**32**	**65,648**	**17**	**2.9**
	5,314	971	971	1,000	45	65,648	18	
	1,466	279	77	9	27	14,963	3	
Settled	**2,049**	**520**	**204**	**43**	**39**	**14,963**	**4**	**2.9**
	2,865	968	543	180	51	14,963	5	

Storm 2 (+4:30)								
	6,888	2,330	2,866	9	29	77,939	15	3.3
Raw	**8,604**	**3,100**	**3,746**	**43**	**40**	**78,483**	**16**	**3.5**
	10,748	4,125	4,895	180	55	79,027	17	3.7
	2,570	1,582	861	18	19	4,114	3	3.5
Settled	**3,390**	**2,191**	**1,294**	**93**	**28**	**4,678**	**3**	**3.6**
	4,470	3,036	1,945	420	41	5,242	3	3.7
Storm 3 (-12:00)								
	4,374	1,223	2,633	90	3	112,212	66	3.6
Raw	**5,555**	**1,749**	**3,465**	**460**	**7**	**115,884**	**70**	**3.9**
	7,055	2,501	4,560	2,000	14	119,556	74	4.3
	2,315	750	1,705	18	5	7,431	19	3.5
Settled	**3,081**	**1,151**	**2,342**	**93**	**10**	**9,031**	**20**	**4.0**
	4,102	1,769	3,216	420	18	10,631	21	4.5
Storm 4 (0:00)								
	1,209	525	173	5	3	72,371	26	1.9
Raw	**1,731**	**858**	**362**	**16**	**7**	**73,512**	**27**	**4.5**
	2,480	1,402	759	42	14	74,653	29	7.0
	906	283	281	1	2	4,444	6	3.2
Settled	**1,351**	**525**	**522**	**7**	**5**	**4,603**	**7**	**3.2**
	2,015	974	971	20	12	4,762	8	3.3
Storm 5 (+2:00)								
	18,736	3,129	16,239	90	19	129,307	169	4.8
Raw	**24,325**	**4,061**	**20,844**	**460**	**29**	**130,895**	**176**	**7.2**
	31,583	5,271	26,754	2,000	42	132,483	182	9.6
	2,611	1,195	7,615	4	15	4,465	15	5.3
Settled	**3,439**	**1,714**	**9,500**	**15**	**23**	**6,884**	**17**	**5.5**
	4,528	2,458	11,851	42	35	9,302	20	5.7

Meeting of the Waters Creek

	Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringens spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background 1								
	2,370	170	213	8	8	9,993	5	
Raw	**2,768**	**206**	**255**	**42**	**14**	**9,993**	**6**	
	3,234	250	304	184	23	9,993	7	
	2,076	93	182	2	5	3,570	2	
Settled	**2,412**	**119**	**220**	**11**	**10**	**3,570**	**3**	
	2,804	152	265	39	18	3,570	4	
Background 2								
	8,060	308	234	9	15	10,316	5	2.9
Raw	**9,307**	**360**	**278**	**44**	**23**	**10,316**	**7**	**3.0**
	10,747	421	330	185	34	10,316	9	3.0
	4,996	201	150	4	8	2,742	1	2.9
Settled	**5,798**	**240**	**184**	**20**	**14**	**2,742**	**2**	**3.0**
	6,729	288	225	77	24	2,742	3	3.1
Background 3								
	1,144	32	53	1	4	10,384	0	2.8
Raw	**1,437**	**58**	**86**	**9**	**8**	**10,718**	**2**	**2.9**
	1,803	105	141	38	16	11,051	6	3.0
	721	485	96	5	1	2,189	0	2.7
Settled	**900**	**613**	**142**	**23**	**4**	**2,366**	**1**	**2.9**
	1,123	774	267	94	10	2,542	1	3.2
Storm 1 (+8:00)								
	4,767	1,170	847	24	5	109,645	58	5.2
Raw	**6,623**	**1,987**	**1,513**	**112**	**11**	**109,645**	**65**	**5.3**
	8,973	3,090	2,517	420	19	109,645	72	5.3
	2,437	620	610	5	9	12,312	1	5.1
Settled	**3,643**	**1,180**	**1,177**	**22**	**16**	**12,312**	**3**	**5.2**
	5,230	2,060	2,067	77	26	12,312	5	5.3
Storm 2 (+6:00)								
	6,076	851	1,534	90	110	61,645	13	
Raw	**7,612**	**1,281**	**2,132**	**460**	**132**	**61,645**	**17**	**3.5**
	9,537	1,929	2,965	2,000	157	61,645	21	
	7,195	797	1,042	90	67	46,960	10	
Settled	**8,982**	**1,213**	**1,523**	**460**	**84**	**46,960**	**12**	**3.6**
	11,212	1,845	2,226	2,000	103	46,960	14	
Storm 3 (+0:45)								
	13,225	3,090	2,670	90	59	131,368	140	4.4
Raw	**16,724**	**4,015**	**3,509**	**460**	**75**	**133,788**	**142**	**5.1**
	21,148	5,216	4,612	2,000	94	136,208	144	5.8
	5,751	1,841	1,694	42	65	9,312	17	4.4
Settled	**7,218**	**2,508**	**2,329**	**240**	**82**	**10,208**	**18**	**4.6**
	9,058	3,416	3,201	1,000	102	11,104	19	4.8

Storm 4 (-4:00)								
	7,412	440	2,315	180	5	112,552	351	5.8
Raw	**9,258**	**743**	**3,081**	**1,100**	**11**	**113,195**	**374**	**6.7**
	11,552	1,256	4,102	4,100	20	113,838	397	7.6
	5,725	396	1,385	180	3	14,757	31	3.8
Settled	**7,186**	**683**	**1,950**	**1,100**	**7**	**16,750**	**35**	**4.7**
	9,020	1,180	2,744	4,100	14	18,743	39	5.6
Storm 5 (+8:00)								
	1,683	442	403	42	4	102,854	48	4.2
Raw	**2,315**	**747**	**693**	**240**	**9**	**103,769**	**50**	**4.2**
	3,184	1,261	1,192	1,000	17	104,684	52	4.3
	445	139	283	9	0	3,291	13	3.8
Settled	**750**	**309**	**525**	**43**	**2**	**4,697**	**14**	**3.9**
	1,265	687	974	180	7	6,103	15	3.9
Storm 6 (+0:00)								
	21,503	1,853	4,517	90	27	115,271	109	3.8
Raw	**28,174**	**2,522**	**5,727**	**460**	**38**	**125,774**	**115**	**4.0**
	36,914	3,433	7,262	2,000	52	136,277	121	4.2
	9,926	1,377	2,315	18	26	12,139	26	3.5
Settled	**12,403**	**1,939**	**3,081**	**93**	**37**	**19,133**	**31**	**3.8**
	15,499	2,732	4,102	420	51	26,127	36	4.1

Booker Creek

	Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringens spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background 1								
	752	426	55	2	31	4,570	7	4.0
Raw	**855**	**596**	**74**	**14**	**43**	**4,570**	**9**	**4.1**
	973	834	101	57	57	4,570	11	4.2
	511	115	22	1	22	2,370	4	4.6
Settled	**585**	**203**	**34**	**5**	**32**	**2,370**	**6**	**5.0**
	671	358	53	50	45	2,370	8	5.5
Background 2								
	828	699	72	4	2	2,917	0	3.8
Raw	**941**	**920**	**95**	**19**	**6**	**3,010**	**2**	**4.1**
	1,069	1,211	124	79	13	3,103	4	4.3
	641	641	97	0	1	1,106	0	3.8
Settled	**730**	**730**	**123**	**3**	**3**	**1,274**	**2**	**4.1**
	832	832	156	12	9	1,442	4	4.4
Background 3								
	1,493	28	170	18	11	9,500	3	6.3
Raw	**1,903**	**52**	**234**	**93**	**18**	**10,339**	**5**	**6.3**
	1,123	97	322	420	28	11,179	6	6.3
	1,195	32	134	5	21	1,602	0	6.4
Settled	**1,503**	**57**	**189**	**23**	**31**	**2,791**	**1**	**6.6**
	1,890	104	267	94	44	3,980	1	6.8
Storm 1								
	2,494	105	360	42	19	65,648	14	
Raw	**3,298**	**254**	**633**	**240**	**28**	**65,648**	**14**	**4.9**
	4,361	612	1,115	1,000	41	65,648	15	
	1,696	173	206	9	24	14,963	0	
Settled	**2,331**	**362**	**412**	**43**	**34**	**14,963**	**1**	**5.0**
	3,203	759	826	180	48	14,963	2	
Storm 2								
	107,280	20,091	11,047	180	40	118,444	83	8.7
Raw	**140,872**	**26,216**	**13,848**	**1,100**	**53**	**120,524**	**84**	**9.0**
	184,983	34,209	17,359	4,100	69	122,604	85	9.2
	31,261	14,910	8,270	90	44	6,395	12	9.4
Settled	**41,067**	**19,010**	**10,314**	**460**	**58**	**7,187**	**13**	**9.7**
	53,950	24,238	12,862	2,000	75	7,979	14	10.0
Storm 3								
	8,683	2,244	3,808	180	5	140,714	243	0.0
Raw	**10,830**	**2,996**	**4,876**	**1,100**	**11**	**142,777**	**244**	**6.0**
	13,509	4,000	6,243	4,100	20	144,840	245	9.5
	4,494	1,889	2,709	42	7	10,046	21	0.0
Settled	**5,700**	**2,566**	**3,557**	**240**	**13**	**10,997**	**27**	**3.4**
	7,229	3,489	4,670	1,000	22	11,948	33	8.7

Storm 4								
	3,845	1,654	2,200	90	9	89,321	32	2.1
Raw	**4,929**	**2,280**	**2,943**	**460**	**16**	**95,204**	**38**	**4.6**
	6,296	3,142	3,937	2,000	26	101,087	44	7.0
	2,147	664	611	17	2	1,290	12	5.5
Settled	**2,879**	**1,040**	**971**	**75**	**6**	**5,975**	**13**	**5.8**
	3,860	1,631	1,544	200	13	10,660	14	6.1

Appendix 2

Temperature, pH, and TOC Data

No apparent trends were observed between temperature, pH, or TOC measurements and contaminant concentrations or fraction of contaminant removed. While previous research (Howell et al. 1996, Geldrich et al.1968) has linked temperature and microbial longevity, temperature may not effect microbial concentrations in stormwater runoff.

Contaminant Concentrations and Temperature

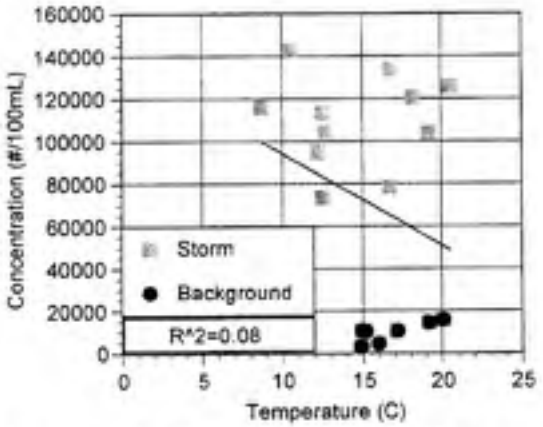


Figure A-1: Particle Concentrations and Temperature

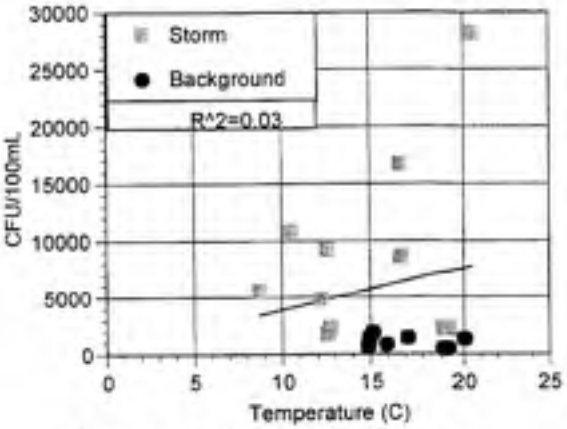


Figure A-2: Fecal Coliform Concentrations and Temperature

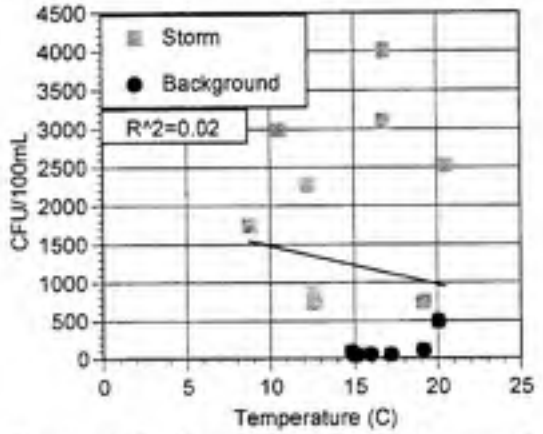


Figure A-3: *E. coli* Concentrations and Temperature

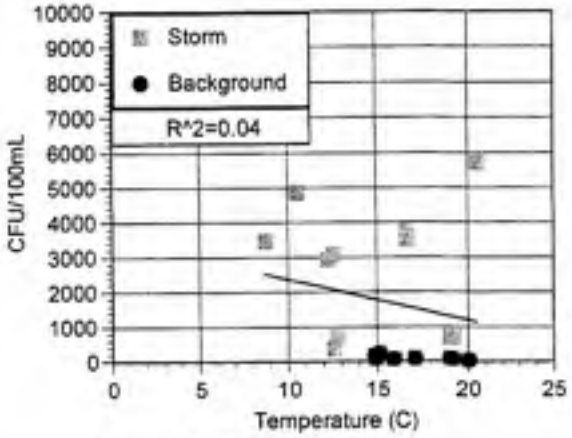


Figure A-4: Enterococci Concentrations and Temperature

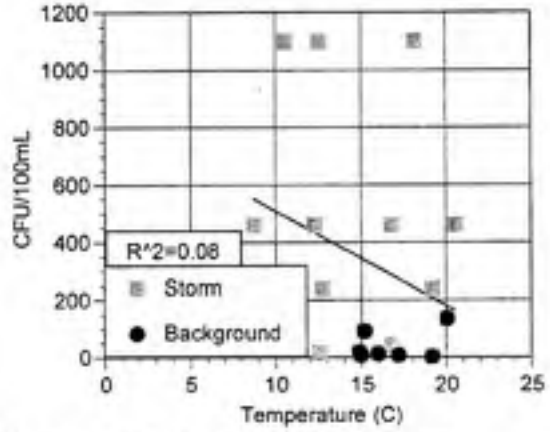


Figure A-5: *Cl. perfringens* Spore Concentrations and Temperature

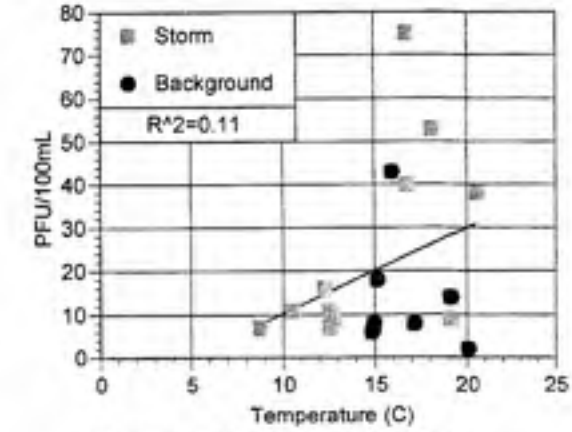
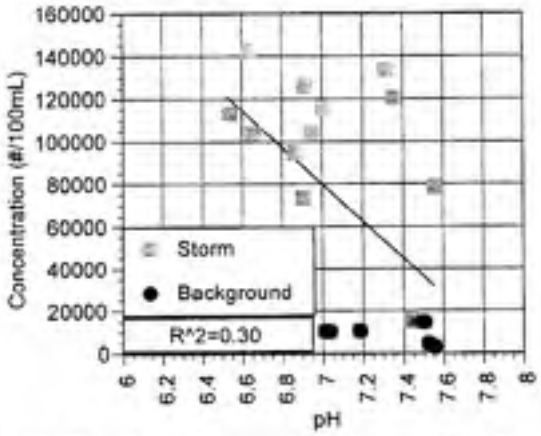


Figure A-6: Total Coliphage Concentrations and Temperature

Contaminant Concentrations and pH



A-7: Particle Concentrations and pH

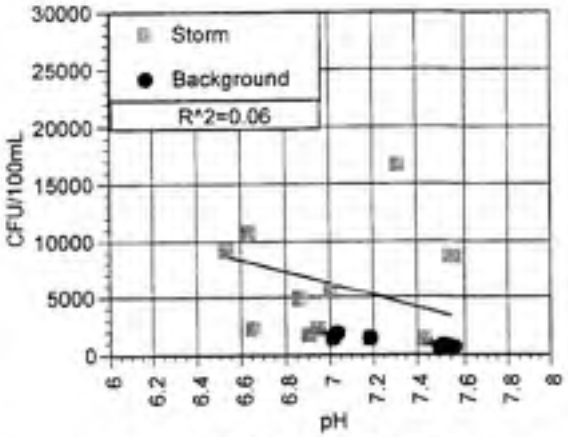
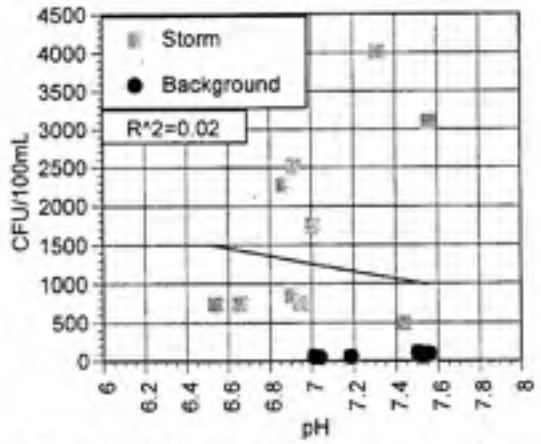


Figure A-8: Fecal Coliform and pH



A-9: *E. coli* Concentrations and pH

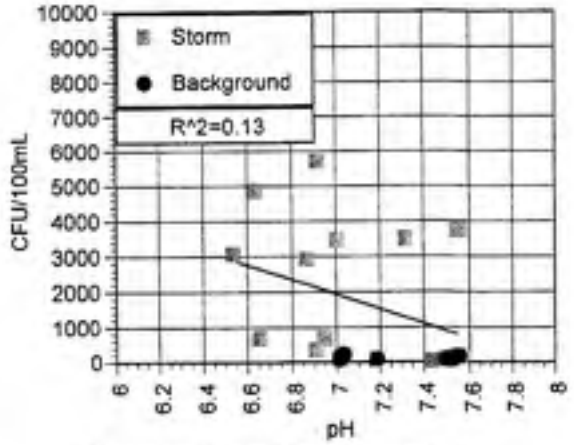
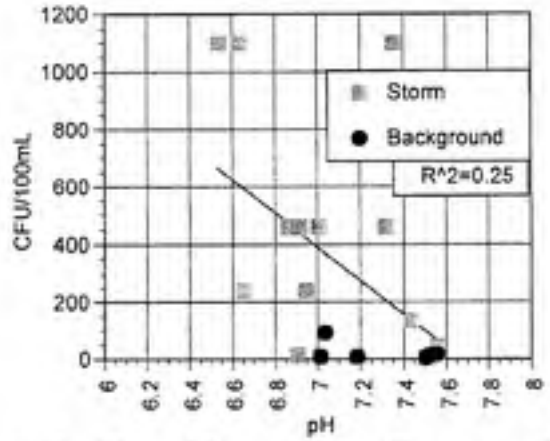


Figure A-10: Enterococci Concentrations and Temperature



A-11: *Cl. perfringens* Spore Concentrations and pH

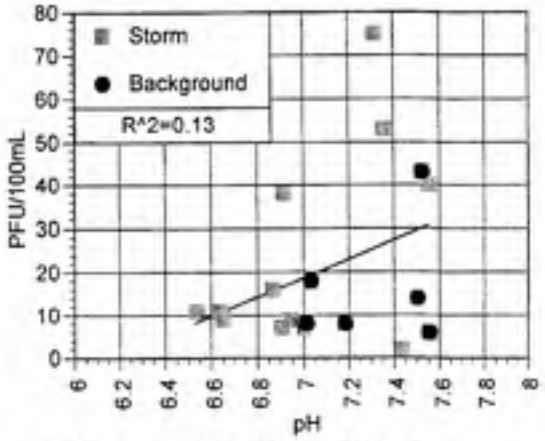
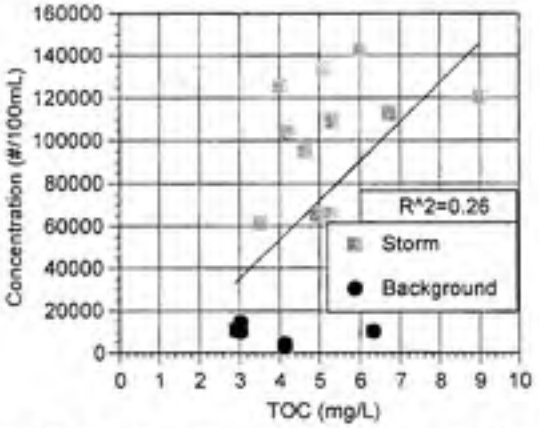


Figure A-12: Total Coliphage Concentrations and pH

Contaminate Concentrations and TOC Concentration



A-13: Particle Concentrations and TOC

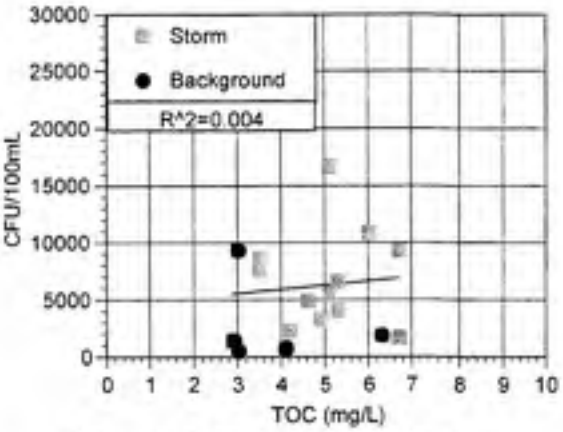
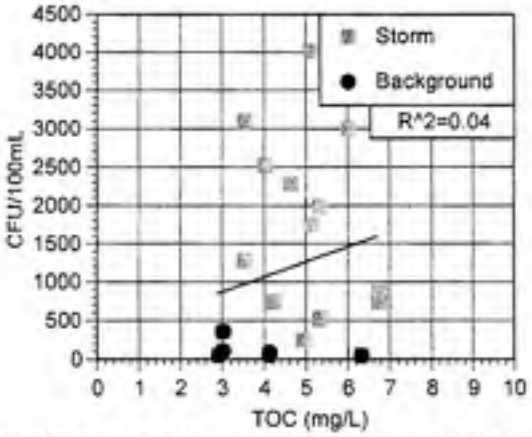


Figure A-14: Fecal Coliform Concentrations and TOC



A-15: *E. coli* Concentrations and TOC

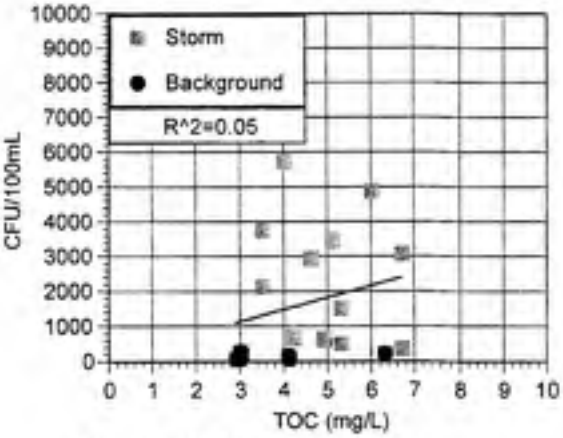
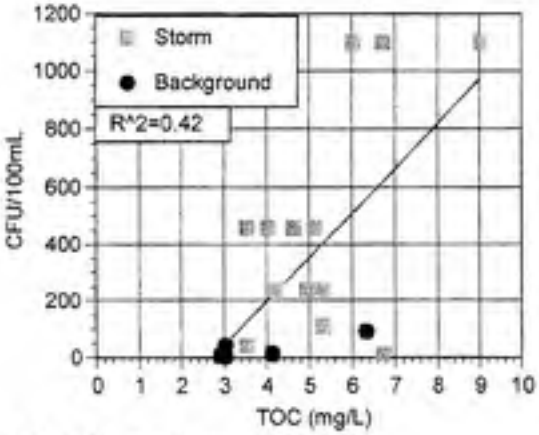


Figure A-16: Enterococci Concentrations and TOC



A-17: *Cl. perfringens* Spores Concentrations and TOC

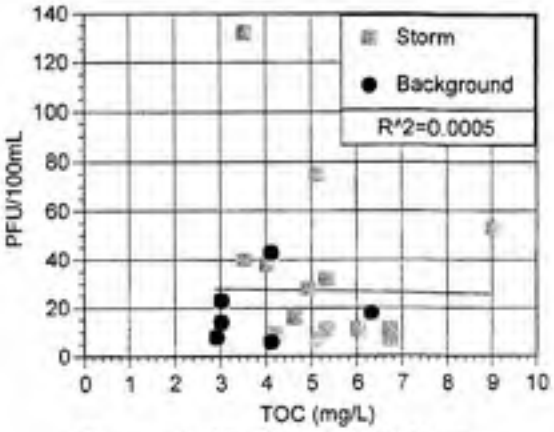
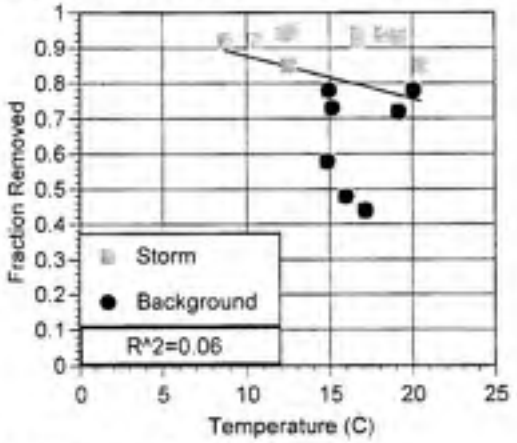


Figure A-18: Total Coliphage Concentrations and TOC

Fraction of Contaminant Removed and Temperature



A-19: Particle Removal and Temperature

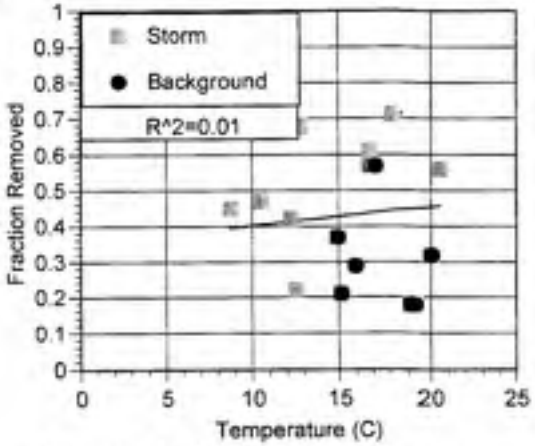
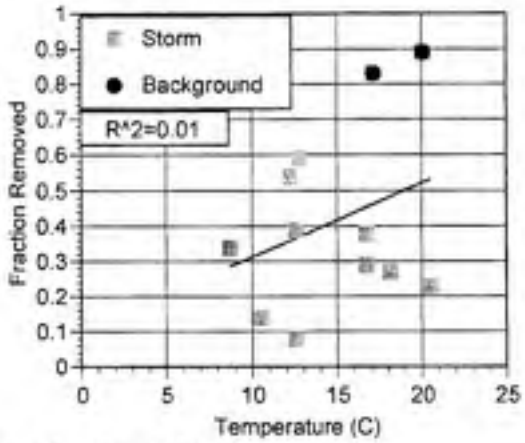


Figure A-20: Fecal Coliform Removal and Temperature



A-21: *E. coli* Removal and Temperature

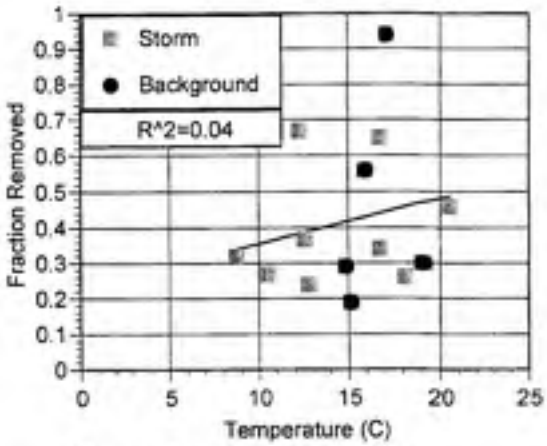
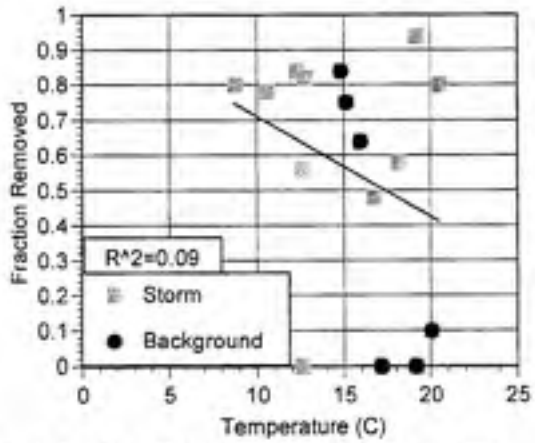


Figure A-22: Enterococci Removal and Temperature



A-23: *Cl. perfringens* Spore Removal and Temperature

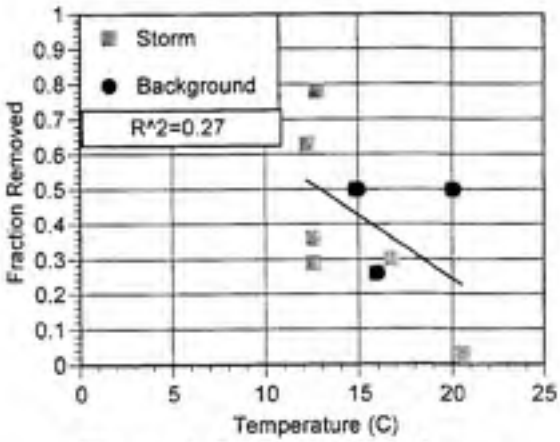
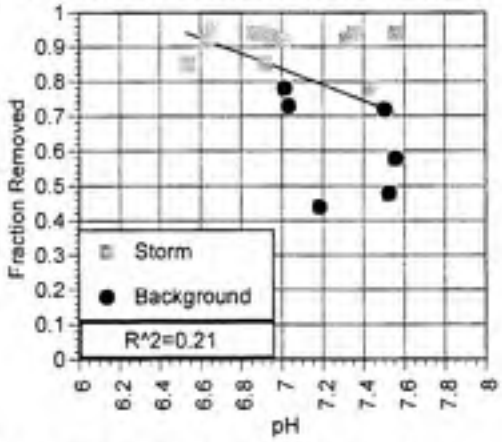


Figure A-24: Total Coliphage Removal and Temperature

Fraction of Contaminant Removed and pH



A-25: Particle Removal and pH

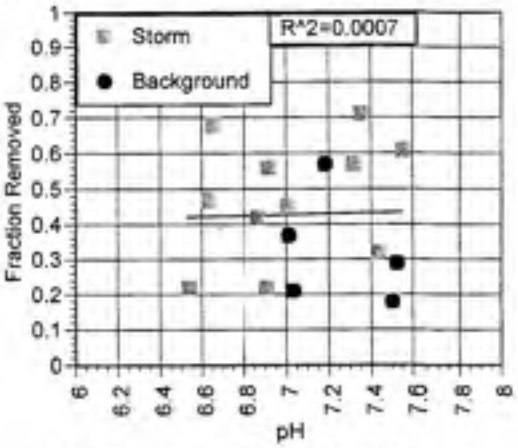
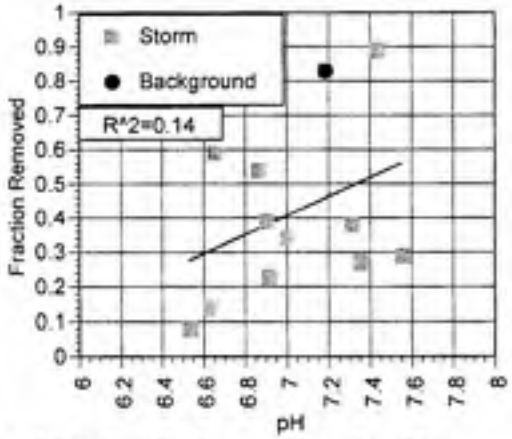


Figure A-26: Fecal Coliform Removal and pH



A-27: *E. coli* Removal and pH

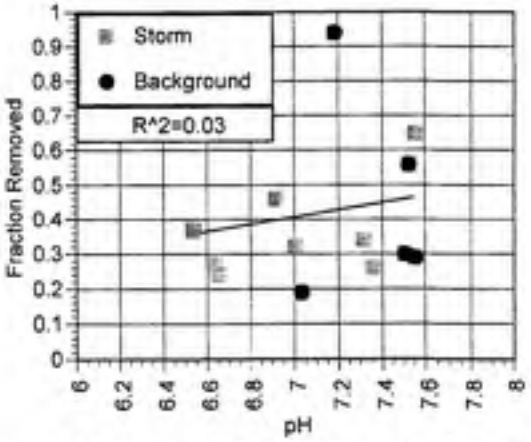
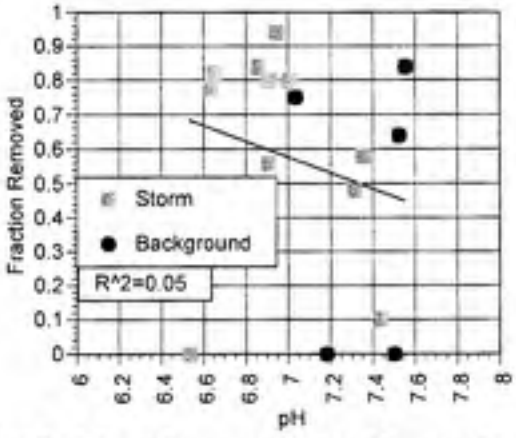


Figure A-28: Enterococci Removal and Temperature



A-29: *Cl. perfringens* Spore Removal and pH

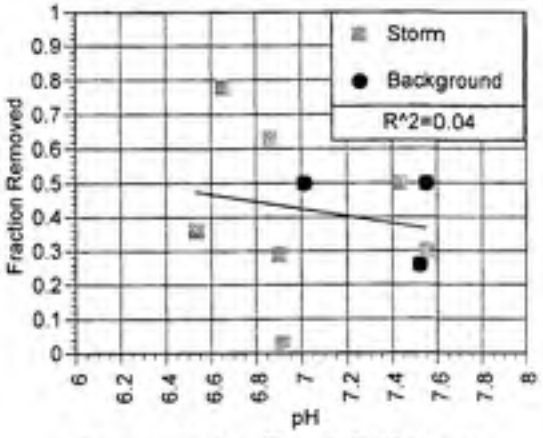


Figure A-30: Total Coliphage Removal and pH

Fraction of Contaminant Removed and TOC

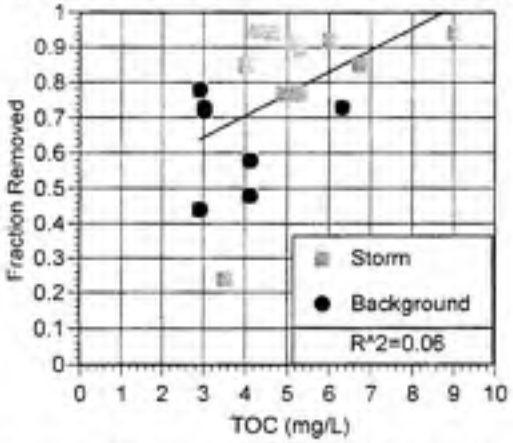


Figure 31: Particle Removal and pH

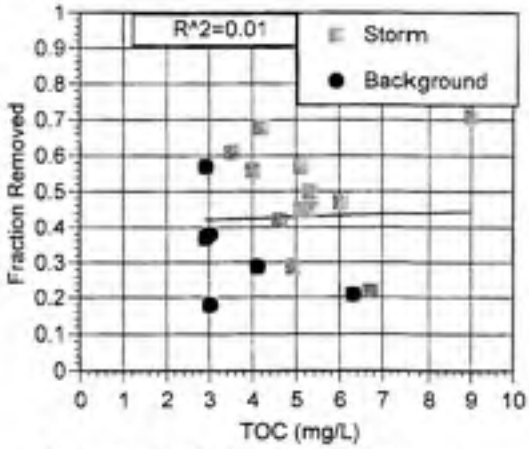


Figure A-32: Fecal Coliform Removal and TOC

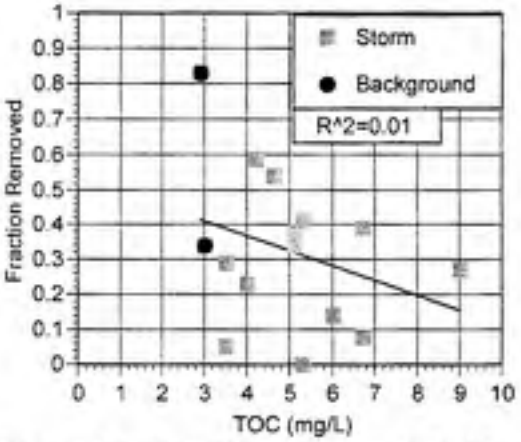


Figure-33: *E. coli* Removal and TOC

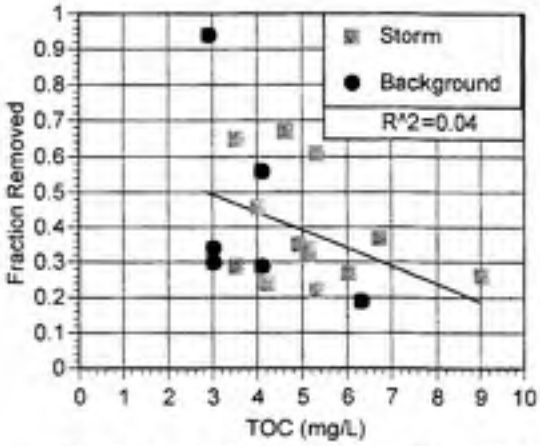


Figure A-34: Enterococci Removal and TOC

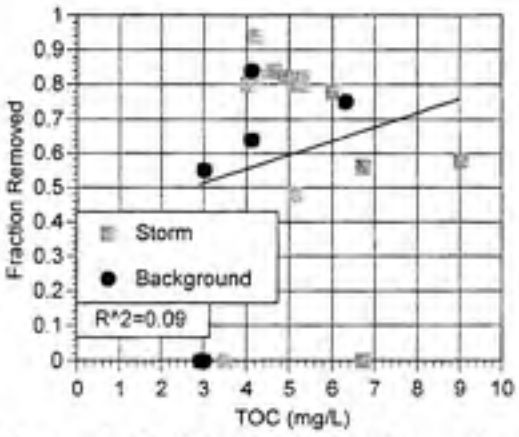


Figure A-35: *Cl. perfringens* Spore Removal and TOC

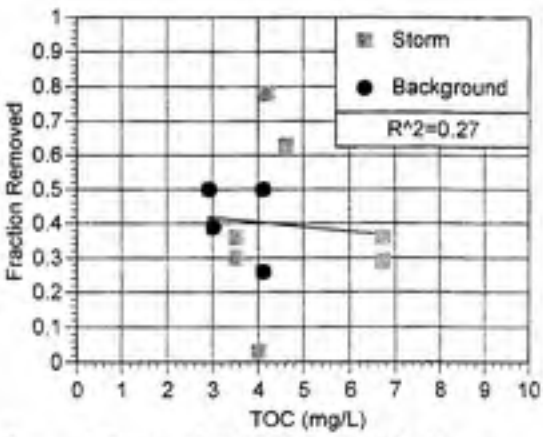


Figure A-36: Total Coliphage Removal and TOC

Appendix 3

The hydrographs for the sampling events at Meeting of the Waters Creek and Eno River are shown below with the time of each sample collected relative to the onset of the storm event. Because of subsequent rainfall, a portion of each hydrograph was interpolated assuming that the stream levels would return to background conditions in 24 hours.

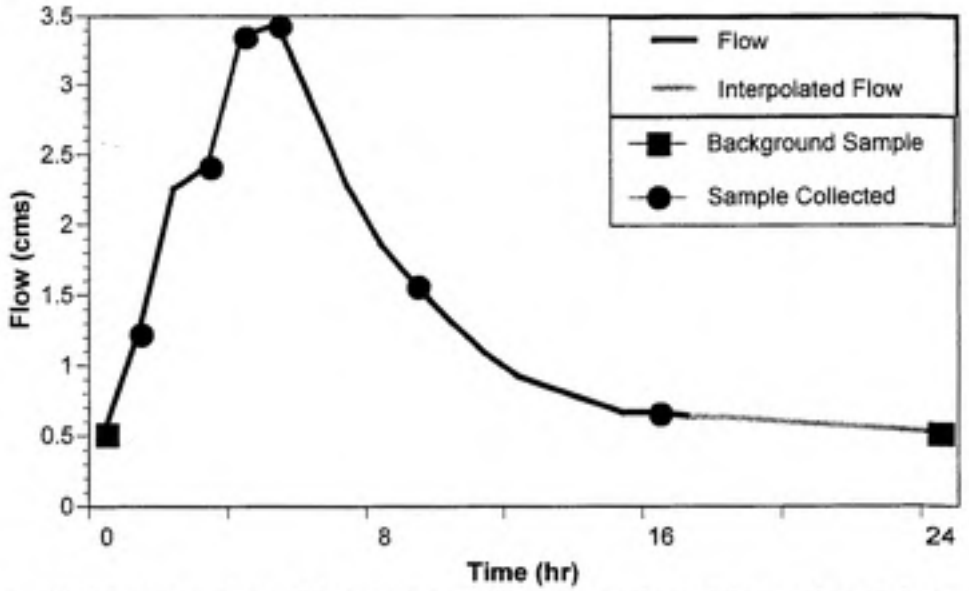


Figure A-37: Meeting of the Waters Creek Hydrograph and Sampling Points

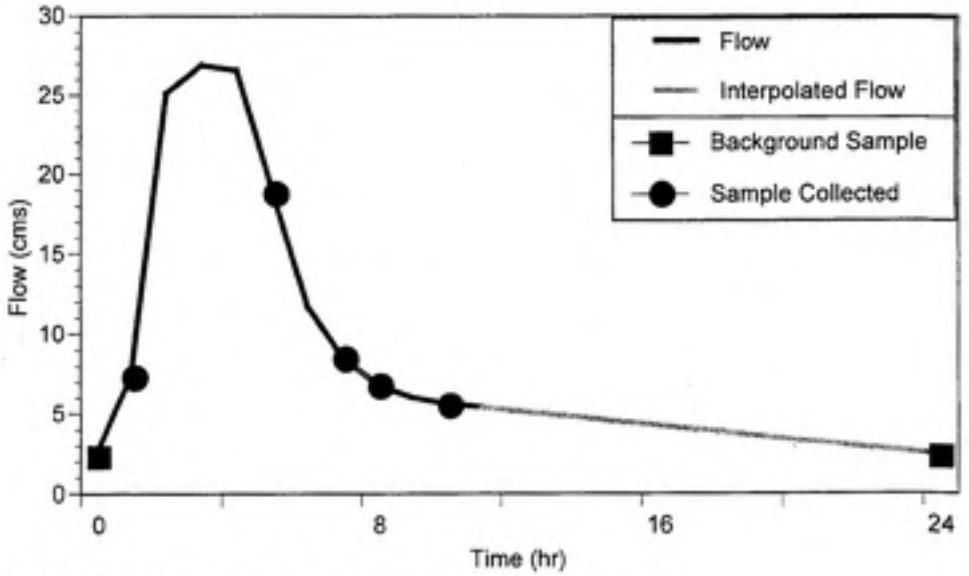


Figure A-38: Eno River Hydrograph and Sampling Points

Appendix 4

Intra storm sampling data are shown for the individual sampling events at Meeting of the Waters Creek and Eno River. Measured values are shown in bold, while 95% confidence interval are shown above (lower confidence value) and below (upper confidence value). The time (t) and stream flow (Q) in cms are also shown for each sample.

Meeting of the Waters Creek

		Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringen Spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		0	37	88	10	6	9,931	2	2.9
t = 16:00	Raw	**4,504**	**208**	**206**	**32**	**15**	**10,342**	**5**	**2.9**
Q = 0.45		9,271	379	325	54	24	10,753	8	3.0
		199	33	138	11	4	2,196	0	2.9
	Settled	**3,037**	**324**	**182**	**18**	**9**	**2,893**	**2**	**3.0**
		5,875	615	226	25	15	3,590	3	3.1
Sample 1		65,817	1,584	3,285	180	2	130,166	607	7.1
t = 16:45	Raw	**84,392**	**2,194**	**4,249**	**1,100**	**6**	**132,683**	**638**	**7.6**
Q = 0.88		108,209	3,038	5,495	4,100	13	135,199	668	8.1
		51,483	485	851	37	3	17,085	25	8.6
	Settled	**66,295**	**804**	**1,281**	**150**	**8**	**23,182**	**26**	**8.7**
		85,369	1,334	1,929	420	16	29,279	27	8.8
Sample 2		51,483	1,369	3,008	420	1	128,262	454	3.5
t = 18:30	Raw	**66,295**	**1,929**	**3,915**	**>1,100**	**4**	**132,643**	**462**	**3.6**
Q = 1.90		85,369	2,719	5,097	-	10	137,023	470	3.7
		19,587	531	1,114	37	5	13,437	57	3.4
	Settled	**25,515**	**866**	**1,613**	**150**	**10**	**18,630**	**62**	**3.4**
		33,236	1,412	2,336	420	18	23,822	66	3.4
Sample 3		17,747	1,493	1,249	90	5	116,429	220	2.5
t = 19:45	Raw	**22,944**	**2,083**	**1,781**	**460**	**10**	**122,409**	**229**	**3.5**
Q = 3.34		29,662	2,905	2,540	2,000	18	128,389	237	4.5
		14	711	573	18	2	16,571	13	2.9
	Settled	**5,865**	**1,101**	**921**	**93**	**6**	**18,385**	**19**	**2.9**
		7,427	1,706	1,481	420	13	20,198	25	2.9
Sample 4		21,503	1,853	4,517	90	27	115,271	109	3.8
t = 21:00	Raw	**28,174**	**2,522**	**5,727**	**460**	**38**	**125,774**	**115**	**4.0**
Q = 3.43		36,914	3,433	7,262	2,000	52	136,277	121	4.2
		9,926	1,377	2,315	18	26	12,139	26	3.5
	Settled	**12,403**	**1,939**	**3,081**	**93**	**37**	**19,133**	**31**	**3.8**
		15,499	2,732	4,102	420	51	26,127	36	4.1
Sample 5		13,557	1,417	5,332	90	35	117,885	63	4.4
t = 1:00	Raw	**17,171**	**1,989**	**6,710**	**460**	**48**	**130,622**	**136**	**4.5**
Q = 1.56		21,748	2,792	8,444	2,000	64	143,358	209	4.6
		18,106	2,244	4,259	9	28	8,601	30	
	Settled	**23,446**	**2,996**	**5,417**	**43**	**39**	**11,780**	**32**	**4.2**
		30,360	4,000	6,890	180	53	14,958	34	

Sample 6		10,853	1,121	3,567	90	20	82,980	41	4.1
t = 9:00	Raw	**13,596**	**1,622**	**4,586**	**460**	**24**	**88,500**	**43**	**4.3**
Q = 0.62		17,032	2,346	5,897	2,000	43	94,021	45	4.5
		6,189	573	2,229	37	15	9,184	23	4.2
	Settled	**7,750**	**921**	**2,978**	**150**	**24**	**9,697**	**24**	**4.3**
		9,705	1,481	3,978	420	34	10,210	24	4.4

Eno River

		Fecal Coliforms (CFU/100mL)	E. coli (CFU/100mL)	Enterococci (CFU/100mL)	Cl. perfringen Spores (CFU/100mL)	Total Coliphage (PFU/100mL)	Particle Concentration (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		465	4	0	0	0	11,794	3	1.9
t = 17:00	Raw	**893**	**63**	**45**	**53**	**7**	**18,664**	**6**	**3.5**
Q = 2.32		1,322	122	92	134	14	25,533	8	5.1
		358	0	3	0	1	2,974	0	2.2
	Settled	**651**	**66**	**36**	**45**	**10**	**4,514**	**3**	**3.4**
		944	135	69	120	19	6,054	6	4.6
Sample 1		18,736	3,129	16,239	90	19	129,307	169	4.8
t = 18:30	Raw	**24,325**	**4,061**	**20,844**	**460**	**29**	**130,895**	**176**	**7.2**
Q = 20.78		31,583	5,271	26,754	2,000	42	132,483	182	9.6
		2,611	1,195	7,615	4	15	4,465	15	5.3
	Settled	**3,439**	**1,714**	**9,500**	**15**	**23**	**6,884**	**17**	**5.5**
		4,528	2,458	11,851	42	35	9,302	20	5.7
Sample 2		15,510	3,476	14,000	42	18	123,361	101	4.9
t = 21:30	Raw	**19,835**	**4,477**	**17,770**	**240**	**27**	**126,836**	**130**	**7.5**
Q = 23:50		25,366	5,767	22,554	1,000	39	130,311	159	4.9
		12,491	2,615	22,032	90	36	5,892	24	7.4
	Settled	**15,744**	**3,443**	**28,902**	**460**	**49**	**9,148**	**32**	**7.5**
		19,844	4,534	37,915	2,000	65	12,405	40	7.6
Sample 3		15,164	3,247	20,507	42	50	112,535	48	7.7
t = 0:00	Raw	**19,359**	**4,202**	**26,795**	**240**	**65**	**118,928**	**154**	**8.0**
Q = 8:44		24,715	5,439	35,011	1,000	83	125,321	260	8.3
		6,586	1,865	11,353	42	73	8,489	19	7.8
	Settled	**8,234**	**2,537**	**14,246**	**240**	**91**	**10,136**	**23**	**7.8**
		10,295	3,450	17,876	1,000	112	11,783	27	7.8
Sample 4		9,073	1,971	8,873	90	66	112,708	65	7.9
t = 1:15	Raw	**11,320**	**2,666**	**11,069**	**460**	**83**	**113,854**	**83**	**7.9**
Q = 6.65		14,125	3,605	13,808	2,000	103	114,999	101	7.9
		3,613	811	6,849	90	64	11,051	18	7.4
	Settled	**4,642**	**1,231**	**8,556**	**460**	**81**	**14,717**	**19**	**7.5**
		5,964	1,867	10,689	2,000	101	18,384	21	7.6
Sample 5		6,976	1,055	6,903	180	47	51,136	73	6.7
t = 3:00	Raw	**8,712**	**1,539**	**8,622**	**1,100**	**61**	**87,942**	**79**	**7.2**
Q = 5.23		10,880	2,245	10,770	4,100	78	124,748	85	7.7
		4,009	763	5,273	18	49	19,995	14	7.1
	Settled	**5,117**	**1,168**	**6,639**	**93**	**64**	**24,836**	**15**	**7.2**
		6,531	1,790	8,358	420	80	29,677	17	7.3